The utilization of organic nutrients in marine phytoplankton with emphasis on coccolithophores

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"Willst du dich am Ganzen erquicken,
so musst du das Ganze im Kleinen erblicken."

Johann Wolfgang von Goethe

Contents

Preface

The anthropogenically caused increase of atmospheric carbon dioxide (CO_2) has many effects on the environmental conditions of the oceans. It changes seawater carbonate chemistry, sea surface temperature, stratification and mixing, light conditions, and nutrient cycling (e.g. Maier-Reimer et al. 1996, Sarmiento et al. 1998, Rost & Riebesell 2004). The environmental conditions and changes have repercussions for the biosphere, influencing succession, distribution, and productivity of phytoplankton (e.g. Hutchinson 1961, Gaedeke & Sommer 1986). In turn, phytoplankton activity can impact the climate by driving many of the oceanic elemental cycles which are connected to the global cycles. In view of the rapid changes in environmental conditions due to the increasing anthropogenic CO_2 emission, the investigation of the biological responses to climate change is the prerequisite to predict the future climate.

Coccolithophores are an important group of phytoplankton, because they influence the oceanic carbon cycle in two ways: The organic carbon pump, influenced by photosynthesis, causes a net draw down of CO_2 from the atmosphere into the ocean and the carbonate counter pump, influenced by calcification, causes a net release of CO_2 to the atmosphere. The flux of CO_2 between the surface ocean and the atmosphere is mainly determined by the relative strength of the pumps (Rost & Riebesell 2004). A changed seawater carbonate chemistry can decrease the calcification rate of coccolithophores (Riebesell et al. 2000, Zondervan et al. 2001, Delille et al. 2005). Also the effects of the combination of some climate-induced changes of environmental conditions were studied (e.g. Sciandra et al. 2003, Leonardos & Geider 2005, Hare et al. 2007, Feng et al. 2008), but mostly on species level as opposed to phytoplankton community level. The investigations on a species level help to understand processes like calcification, but the interactions of species are too complex to predict the response of a phytoplankton community based on experiments on a species level.

Increasing sea surface temperature will enhance stratification, which in turn may reduce the input of inorganic nutrients into the surface layer, prevent organic nutrients from mixing down at the same time, and reduce therewith the ratio of inorganic to organic nutrients. This would favor species, which are able to utilize organic nutrients, but the effects of a changed inorganic to organic nutrient ratio on a phytoplankton community are unknown. The utilization of organic nutrients in general is known for some coccolitho-

phore species (Ietswaart et al. 1994, Palenik & Henson 1997, Waser et al. 1998, Dyhrman & Palenik 2003, Shaked et al. 2006), but only a fraction of the organic nutrients is biologically utilizable (Bronk 2002). Nothing is known about species-specific differences in the utilization of the diverse compounds of the organic nutrients and little is known about strain differences (Dyhrman & Palenik 2003). Knowledge about differences in the utilization of organic nutrients could help to understand succession and distribution patterns of coccolithophores. The ability to utilize organic nutrients is often dependent on trace metals like zinc or nickel which are cofactors of enzymes processing organic nutrient compounds. One study showed a possible Zn-P co-limitation in *Emiliania huxleyi* (Shaked et al. 2006), but nothing is known about other species and other possible co-limitations.

1 Introduction

1.1 Phytoplankton

Phytoplankton are free-floating, unicellular algae, mostly too small to see with the naked eye. This group of plants is taxonomically diverse and consists of at least 20,000 species (Falkowski et al. 2003). Like all plants, phytoplankton is capable of photosynthesis and forms the basis of the marine food web. One prokaryotic and eight eukaryotic major phytoplankton taxa are known, but three phytoplankton clades (dinoflagellates, coccolithophores, and diatoms) dominate the modern ocean (according to Falkowski et al. 2004). The prokaryotic cyanobacteria are suggested to be the majority of phytoplankton in the Proterozoic history (∼1.5 billion years ago) before oxygenic photosynthesis spread via endosymbiosis to eukaryotic clades. The eukaryotic photoautotrophs rived into two lineages, the green algae (and land plants) and the red algae which includes the three dominant phytoplankton clades dinoflagellates, coccolithophores, and diatoms. The dinoflagellates and coccolithophores emerged in the Middle Triassic, whereas the diatoms emerged later in the Mesozoic Era (for detailed information see the review of Falkowski et al. 2004). The phytoplankton could be assigned to different 'functional groups' (according to Falkowski et al. 2003). Functional groups are groups of organisms that are related through common biogeochemical processes, independent from phylogenetic relationship. These biogeochemical processes influence the cycling of elements in the ocean and between the ocean and the atmosphere. Functional groups of phytoplankton include diazotrophs, silicifiers, calcifiers and dimethylsulfide (DMS) producers. Diazothrophs (or N_2 fixers) reduces N_2 to ammonium-N (and then to organic molecules), silicifiers converts soluble silicic acid to solid hydrated amorphous opal, calcifiers converts dissolved inorganic carbon and calcium to solid-phase calcite, and DMS producers synthesis dimethylsulfoniopropionate (DMSP) which is enzymatically cleaved to DMS by bacteria and phytoplankton (for further information see the review of Hood et al. 2006).

Different taxa of phytoplankton have different demands on the environmental conditions. Nutrient availability, light level, and temperature are only some conditions which decide competitive advantage and distribution of the different phytoplankton species (e.g. Hutchinson 1961, Gaedeke & Sommer 1986). The continuously changing physical, chemical, and biological factors of the ocean cause a continuous change of different species

and algae groups. This process of continuous community reorganization within one water mass is termed succession (Smayda 1980). The seasonal succession of the three dominant phytoplankton clades starts in general with a diatom spring bloom which is replaced by a summer dinoflagellate or coccolithophore community (for detailed information about succession see Smayda (1980)).

Diatoms contribute to the functional group of silicifiers, they build opal frustules (Martin-Jézéquel et al. (2000)). Diatoms account for 40% of the total primary production in the ocean and occur in all oceans, but mostly in sub-polar, and polar zones, coastal zones and nutrient-rich upwelling regions (Sarthou et al. 2005 and references therein). Diatoms often bloom in spring and appear to dominate as long as silicate concentrations are high (Egge & Aksnes 1992, Egge & Heimdal 1994).

Coccolithophores contribute to the functional groups of calcifiers and of DMS producers. The majority of coccolithophore species occurs in the warmer regions (Lalli & Parsons 1994b), although some species thrive in colder regions (e.g. *Emiliania huxleyi* and *Coccolithus pelagicus*) (Winter et al. 1994). *E. huxleyi* is the best studied coccolithophore due to the easy culturing of this species, the easy observation of blooms by remote sensing techniques (Brown & Yoder 1994, Balch et al. 2001), and its status as a model organism (Westbroek et al. 1993). This species is a good competitor for phosphate, but does not grow well under low nitrate concentrations (Egge & Heimdal 1994). To bloom *E. huxleyi* needs an irradiance above 20 μmol photons m^{-2} s^{-1} and temperature above 10°C (Egge & Heimdal 1994 and references therein). For further and detailed information about coccolithophores the reader is referred to Thierstein & Young (2004).

Although we have some general ideas on specific requirements of some species, we are far from understanding phytoplankton distribution and succession in today's ocean (Rost & Riebesell 2004). Moreover, anthropogenic carbon dioxide (CO_2) emission and the associated global warming rapidly change some of the environmental factors in the ocean. These expected changes make it especially important to understand which parameters drive the succession of phytoplankton species. The influence of some changing parameters on a phytoplankton assemblage of the northeast Atlantic is described in studies I and II of this thesis.

1.2 Marine carbon cycle, sea surface temperature, and the impact of phytoplankton

Phytoplankton species influence the biogeochemical cycling of different elements like carbon and nitrogen in different degrees due to their association to different functional groups. The ocean influences the air/sea CO_2 exchange and represents the largest reservoir of carbon on earth with 60 times more carbon as the atmosphere and 20 times more carbon as the terrestrial biosphere/soil compartment (Denman et al. 2007). Gaseous CO_2 dissolves in seawater and is hydrated to form carbonic acid (H_2CO_3) which dissociates to bicarbonate (HCO_3^-), carbonate (CO_3^{2-}) and protons (H^+). The sum of all inorganic carbon species is called dissolved inorganic carbon (DIC). The proportion of these carbon species varies as a function of pH. In present day seawater (pH of 8.2) the ratio of HCO_3^-:CO_3^{2-}:CO_2 is about 90:9:1 (Wolf-Gladrow et al. 1999). For detailed information on the carbonate system see Zeebe & Wolf-Gladrow (2001).

The marine carbon cycle and the air/sea CO_2 exchange is determined by the carbon pumps (Fig. 1). One physical pump (the so-called 'solution pump') and two biological pumps (the 'organic carbon pump' and the '$CaCO_3$ counter pump') transport carbon into the deep (Volk & Hoffert 1985). The '$CaCO_3$ counter pump' is called 'counter pump', because $CaCO_3$ precipitation in surface waters produces CO_2 according to the following equation:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O \quad (1)$$

The consumption of HCO_3^- shifts the CO_2 concentration gradient between the ocean and the atmosphere and caused a net release of CO_2 to the atmosphere (Holligan et al. 1993). The flux of CO_2 between the surface ocean and the atmosphere is mainly determined by the strength of both pumps (Rost & Riebesell 2004), represented by the ratio of inorganic to organic carbon export in the ocean, the rain ratio (Archer et al. 2000).

The anthropogenically caused increase of atmospheric CO_2 has many effects on the biogeochemical properties of the ocean. The marine CO_2 concentration increases and changes the carbonate system of the ocean. Because all parameters of the carbonate system are interdependent, a change in the atmospheric CO_2 concentration alters the ratio of carbon species and also the pH of the seawater. Changing CO_2 concentrations seem to influence phytoplankton growth directly. Elevated CO_2 concentrations caused an increase of the primary production in the North Atlantic (Hein & Sand-Jensen 1997) and caused an in-

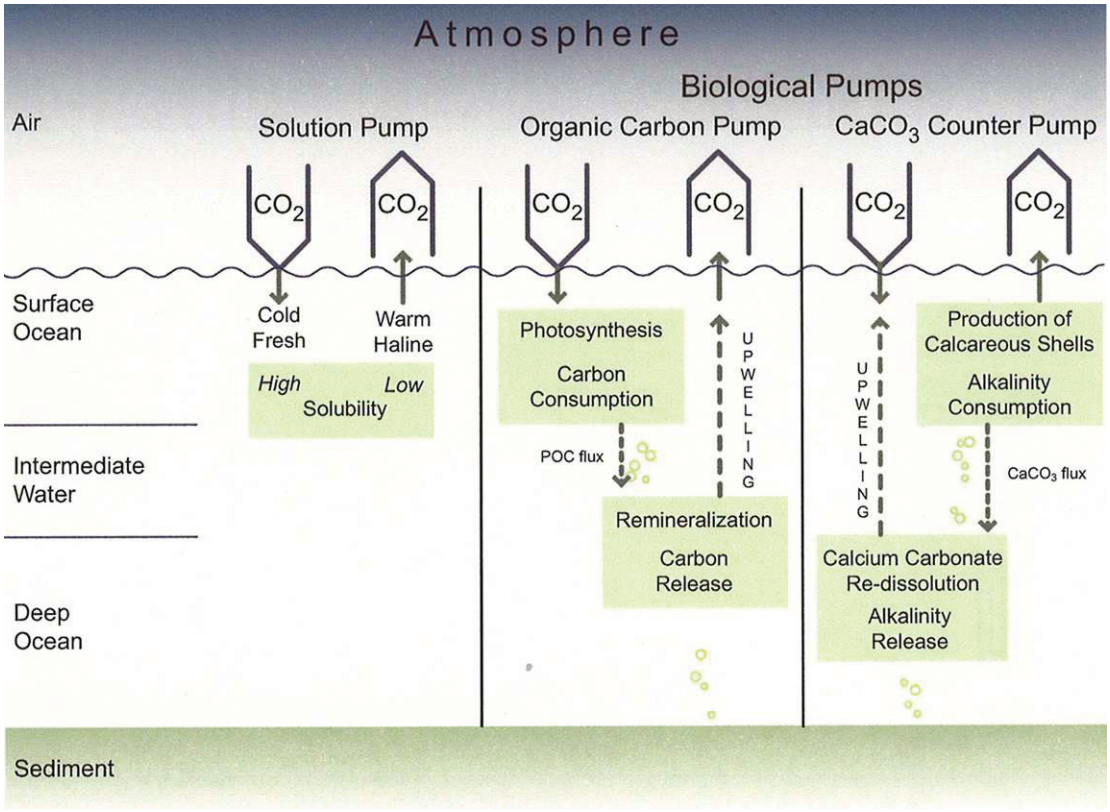

Figure 1: Three main ocean carbon pumps govern the regulation of natural atmospheric CO_2 (Heinze et al. 1991)

creased particulate organic carbon (POC) production by the coccolithophores *Emiliania huxleyi* and *Gephyrocapsa oceanica* (Riebesell et al. 2000). But this finding does not hold for all coccolithophore species (Langer et al. 2006) and the effect of CO_2 on phytoplankton growth is still under discussion (Wolf-Gladrow et al. 1999 and references therein).

Because carbon assimilation by phytoplankton is limited by the catalytic inefficiency of the enzyme Ribulose-1,5 bisphosphate carboxylase/oxygenase (RubisCO) under present atmospheric conditions, algae have developed differently efficient CO_2 concentrating mechanisms (CCM's) that enhance the intracellular CO_2 concentration at the site of carboxylation. Rates of carbon fixation of some species are at or close to CO_2-saturation at present day CO_2 levels (Raven & Johnston 1991, Burkhardt et al. 2001, Rost et al. 2003), but some species seem to be well below saturation at these levels (Paasche 1964, Nielsen 1995, Rost et al. 2003). Consequently some species may benefit more from the present increase in atmospheric CO_2 compared to others. The experiment of study I investigate the effect of an enhanced CO_2 concentration on a mixed phytoplankton community in the northeast Atlantic.

The anthropogenic CO_2 emission also increases the global temperature and the sea surface temperature (SST) (Bopp et al. 2001). Increased temperatures accelerate metabolic reactions and increase the growth rates of phytoplankton, but each phytoplankton species (and sometimes even strains) have their optimum temperature, they are adapted to (Eppley 1972). An increased SST may drive some species either toward their optimum temperature or outside their temperature range and, hence, may change the phytoplankton composition. The effect of a changed SST on community structure is studied on a mixed phytoplankton community from the northeast Atlantic (study I of this thesis).

1.3 Nitrogen and phosphorus in the ocean

Temperature gradients in the water column affect stratification and mixing of the different water masses and therewith the exchange of nutrients between deeper, nutrient-rich water masses and the euphotic zone. An enhanced stratification of the ocean due to increased SST will reduce the input of inorganic nutrients from the deep to surface waters (Rost & Riebesell 2004), but will prevent organic nutrients from mixing down at the same time. This will decrease the concentration of inorganic nutrients, increase the concentration of organic nutrients, and decrease the ratio of inorganic to organic nutrients in the euphotic zone. The ratio of inorganic to organic nutrients reaches from 0.02 to 6 in the open ocean surface and from 0.1 to 9 in coastal zones for nitrogen (according to Berman & Bronk 2003); from 0.01 to 0.75 in the open ocean surface and from 0.02 to 1.7 in the coastal zones for phosphorus (according to Karl & Björkman 2002). Assuming a decrease of around 6% of phosphate concentration in the future (Bopp et al. 2001) and a constant concentration of dissolved organic phosphorus, an initial ratio of inorganic to organic phosphorus of 0.75 may decrease to 0.71. Phytoplankton which is able to utilize organic nutrients will probably be more competitive under the changed nutrient concentrations and ratio. The effect of a changed inorganic to organic nutrients ratio on a phytoplankton community is investigated in study II.

Nitrogen occurs in several forms in the ocean. The inorganic compounds (dissolved inorganic nitrogen, DIN) are gaseous nitrogen (N_2), nitrate, nitrite, and ammonium. Gaseous nitrogen is exchanged with the atmosphere and the biological nitrogen fixation adds this nitrogen as reactive compounds to the sea (Fig. 2a). Nitrate and nitrite enter the

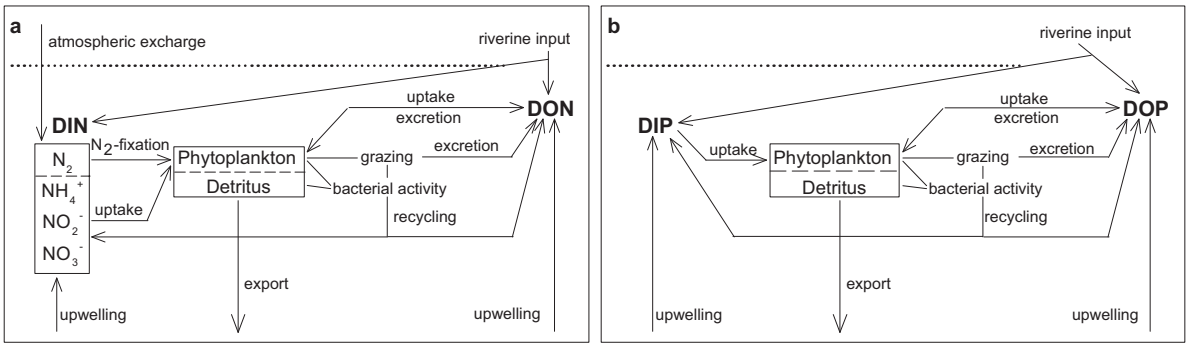

Figure 2: The cycle of nitrogen (a) and phosphorus (b) in the euphotic zone. DIN dissolved inorganic nitrogen, DON dissolved organic nitrogen, DIP dissolved inorganic phosphorus, and DOP dissolved organic phosphorus. Figures modified according to Lalli & Parsons (1994a)

surface waters mostly by upwelling and riverine input and are usable as nitrogen sources for phytoplankter. The dissolved organic nitrogen (DON) consists of many different compounds. DON and ammonium enter the surface waters by upwelling and riverine input and are generated by excretions of plankton and marine animals or the remineralization of organic matter (Fig. 2a). The cycling of phosphorus in the upper water column is similar to the cycling of nitrogen with the exception of the N_2-fixation, the phosphorus cycle has no correspondent and the entry of phosphorus is completely dependent from upwelling and riverine input (Fig. 2b). The export of biomass into the deep removes nitrogen and phosphorus from the upper water column until nitrogen and phosphorus are welled up again in different compounds (Fig. 2). For detailed information about the cycling of nitrogen and phosphorus in the ocean see Cembella et al. (1984), Föllmi (1996), Benitez-Nelson (2000), Berman & Bronk (2003), and Capone & Knapp (2007).

Phytoplankton growth depends among other factors on the availability of nutrients (e.g. Dugdale 1967, Eppley et al. 1969). Every phytoplankton cell needs nitrogen and phosphorus to grow and build up biomass. The availability of nitrogen and phosphorus in the upper water column is controlled by vertical mixing, river inflow, nitrogen-fixation, and the recycling of organic matter. But nutrients not only influence the growth of the phytoplankton, the nutrient availability also change the calcification. *Emiliania huxleyi* shows enhanced calcification under nutrient limitation (Merrett et al. 1993, Nimer & Merrett 1993, Paasche & Brubak 1994, Paasche 1998, Berry et al. 2002). The influence of nutrient limitation on the calcification rate of *Coccolithus pelagicus* is investigated in study III.

Studies concerned with the response of phytoplankton to nutrient availability traditionally focus on nitrate and orthophosphate. However, nitrate and orthophosphate are not the only nitrogen and phosphorus sources in the ocean. The DON supplies around 63% of the total dissolved nitrogen (TDN) (Bronk 2002) and the DOP up to 75% of the total dissolved phosphorus (TDP) (Benitez-Nelson 2000 and references therein) in the ocean. DON concentrations reach ~8 μmol N L^{-1} in the Pacific and Atlantic and up to 52.5 μmol N L^{-1} in the coastal zones (Berman & Bronk 2003 and references therein). DOP concentrations reach 0.285 μmol P L^{-1} in the Pacific, 0.5 μmol P L^{-1} in the Atlantic, and up to 2 μmol P L^{-1} in the coastal zone (Karl & Björkman 2002 and references therein). Two of the largest fractions of the DON are urea and the free and combined amino acids (Antia et al. 1991 and references therein). The amino acids are divided in non-polar, polar, acidic, and basic amino acids (Antia et al. 1991 and references therein). DOP consists mostly of monophosphate esters (55-77%) and nucleotides and nucleic acids (23-45%) (Benitez-Nelson 2000 and references therein).

Although DON and DOP could occur in biologically usable concentrations, presumably only a fraction is biologically utilizable (Bronk 2002). Many diatoms are also able to utilize organic nutrients like urea, amino acids, phosphomonoesters, and phosphate diesters (García Ruiz et al. 1997, Peers et al. 2000, Fan et al. 2003, Lomas 2004, Yamaguchi et al. 2005, Rees & Allison 2006), whereas only the utilization of phosphomonoesters via the enzyme alkaline phosphatase is known for *Phaeocystis* (Admiraal & Veldhuis 1987, van Boeckel 1991). The availability of different organic nitrogen sources changed a phytoplankton assemblage in a lake and indicated that different algal species have varying capabilities to utilize these sources (Berman & Chava 1999). The differences in the utilization of organic nutrients could supply new properties of biogeographic distribution patterns and the succession of phytoplankton species. Future studies on distribution and concentration of the different organic nutrient sources are necessary to combine the physiological studies with natural conditions in the ocean. Which compounds of the organic nutrients are usable by the phytoplankton seems to be quite species-specific (Dyhrman & Palenik 2003). The ability of three coccolithophore species to utilize organic nutrients is investigated in study IV of this thesis.

Urea, a small, neutral molecule, can diffuse passively into the cell, but urea is also taken up by cells via an active transport system (Antia et al. 1991 and references therein). Once

inside the cell urea is cleaved into CO_2 and ammonium either by the enzyme urease (EC 3.5.1.5) or by the ATP:urea amidolyase (UALase) reaction (EC 6.3.4.6 and EC 3.5.1.13) (Leftley & Syrett 1973). The UALase reaction was only detected in some orders of green algae, whereas in some representatives of the other algae classes only the enzyme urease was detected (Bekheet & Syrett 1977). Urease of higher plants (Dixon et al. 1975) and bacteria (Mobley & Hausinger 1989) requires nickel for its catalytic activity and could be therewith Ni-N co-limited (Saito et al. 2008). The utilization of urea was shown in several phytoplankton species of all classes (McCarthy 1972, Antia et al. 1991, Waser et al. 1998). Also, urease was detected in several species (Collier et al. 1999, Peers et al. 2000, Fan et al. 2003), but not in coccolithophores. The co-limitation of nitrogen and nickel was also shown for some phytoplankton species (Oliveira & Antia 1986, Price & Morel 1991), but it is not known whether coccolithophores need nickel to grow on urea. This question was addressed in a comparative study on three coccolithophore species (study V of this thesis).

1.4 Outline of the thesis

This thesis investigates some effects of CO_2-induced changes of environmental conditions on marine phytoplankton on the single species as well as on the community level.

Study I investigates the response of a North Atlantic plankton assemblage to combined changes of CO_2 concentration and temperature. The effects of CO_2 concentration and temperature on phytoplankton composition, biomass build-up, and particulate elements are discussed in view of potential climatic feedbacks.
Study II reports the effect of a changed ratio of inorganic to organic nutrients on a phytoplankton assemblage from the North Atlantic. The results are interpreted in an ecological and biogeochemical context.
Study III investigates the effects of nitrogen limitation on the calcification of *Coccolithus pelagicus*. The results are interpreted in an ecological and biogeochemical context.
Study IV investigates the utilization of organic nutrients in three coccolithophore species in more detail. The aim of this study is to assess species-specific differences in the utilization of dissolved organic nitrogen and phosphorus compounds. The apparent differences of the three species are discussed in view of competition and distribution of the species.
Study V addresses the question whether coccolithophores need nickel to grow on urea. The species-specific differences are discussed in regard to a potential nickel-nitrogen co-limitation in the ocean.

Each study is presented in the format of a scientific paper. In a concluding discussion main results of this study are summarized and discussed with respect to the possible consequences of anthropogenic CO_2 emission and the associated change of several parameters in the ocean on a phytoplanktonic assemblage and, in more detail, on coccolithophores. A perspective on future research is given at the end.

2 Studies

Declaration on the contribution of each study

Study I: This experiment was conceived and conducted by Yuanyuan Feng and David A. Hutchins. Samples of POC was taken and measured by me. The data were interpreted and the study was written mainly by Yuanyuan Feng together with Clinton E. Hare, Karine Leblanc, Julie M. Rose, Yaohong Zhang, Giacomo R. DiTullio, Peter A. Lee, Steven W. Wilhelm, Janet M. Rowe, Jun Sun, Nina Nemcek, Celine Guenguen, Uta Passow, Ina Benner, and David A. Hutchins.

Study II: The experiment was conceived and conducted by Gry Mine Berg and me. Samples were taken and measured in teamwork by Gry Mine Berg, Claudia Sprengel, Karine Leblanc, Yuanyuan Feng, Janet M. Rowe, and me. The data were interpreted and the study was written mainly by me together with Uta Passow, Gry Mine Berg, Claudia Sprengel, Karine Leblanc, Yuanyuan Feng, David A. Hutchins, Janet M. Rowe, and Steven W. Wilhelm.

Study III: The experiment was conceived and conducted by me, also the taken and measuring of the samples. The data were interpreted and the study was written mainly by me together with Gerald Langer.

Study IV: The experiment was conceived and conducted by me as well as the taken and measuring of samples. Data were interpreted and the study written mainly by me together with Uta Passow.

Study V: All parts of the study were done by me.

Study I

The Effects of Increased $p\mathrm{CO}_2$ and Temperature on the North Atlantic Spring Bloom: I. The Phytoplankton Community and Biogeochemical Response

INTRODUCTION

Of the many ongoing global anthropogenic change processes, increased atmospheric CO_2 and rising temperatures are likely to have some of the most profound effects on ocean biology and biogeochemistry. Atmospheric CO_2 concentration is currently increasing by about 0.4% per year, and has already increased by about 30% over pre-industrial levels. The predicted atmospheric partial pressure of CO_2 ($p\mathrm{CO}_2$) by the end of this century is over 71 Pa (700 parts per million, ppm, Solomon et al. 2007). This will lead to a predicted seawater CO_2 concentration increase of about 30 μmol kg^{-1} and a corresponding seawater pH decrease to about 7.8, roughly 0.3 units lower than today's value (Wolf-Gladrow et al. 1999). At the same time, warming associated with the release of greenhouse gases into the atmosphere has been predicted to raise sea surface temperature (SST) by 1-4°C over the next 100 years (Bopp et al. 2001, Solomon et al. 2007).

These global changes will have major effects on the physiology of marine phytoplankton (Boyd & Doney 2002, Hays et al. 2005). For instance, it has been shown that CO_2 enrichment will significantly influence the photosynthesis, elemental composition and calcification of marine phytoplankton (Riebesell 2004). Furthermore, these effects are taxon-specific, so future phytoplankton community structure and succession should also be influenced (Tortell et al. 2002, Hare et al. 2007). Phytoplankton metabolic activity could be accelerated by elevated temperature within a suitable range (Eppley 1972), therefore rising SST will also have important effects on marine phytoplankton. Labora-

tory studies have predicted that CO_2 enrichment together with rising temperature may have interactive influences on some phytoplankton species (Fu et al. 2007, Hutchins et al. 2007, Feng et al. 2008). However, there is little information available on the effects of simultaneously increased temperature and pCO_2 on natural phytoplankton communities (Hare et al. 2007).

The intense annual North Atlantic spring bloom is one of the most dramatic and predictable biological events in the world ocean (Esaias et al. 1986). Typically, this bloom follows a successional pattern in which initial dominance by diatoms later gives way to nanoplankton, mainly coccolithophores (Lochte et al. 1993). This secondary coccolithophore bloom may be induced by high light conditions (Tyrrell & Taylor 1996), silicate depletion during the early diatom-dominated bloom phase (Sieracki et al. 1993), phosphate becoming more limiting than nitrate (Tyrrell & Taylor 1996, Riegman et al. 1992), low dissolved CO_2 and high carbonate saturation state (Tyrrell & Merico 2004), or some combination of these factors. How community structure, phytoplankton succession, and marine biogeochemical cycles during this annual event will change as a result of increasing future CO_2 concentration and SST is still unknown. The North Atlantic is thus an ideal regime in which to examine experimentally how global changes could drive future shifts in phytoplankton diversity, and the resulting patterns in carbon and nutrient biogeochemistry.

The goals of this study were to investigate the individual and combined effects of increased pCO_2 and temperature on algal community structure, phytoplankton succession, and elemental cycling in the North Atlantic spring bloom area. To do this, we conducted a shipboard continuous culture incubation ('Ecostat', Hare et al. 2005, Hare et al. 2007 using a natural North Atlantic bloom phytoplankton community. Unlike short-term bottle grow-out experiments (days), this shipboard adaptation of laboratory continuous culture system methods offers the possibility of effectively simulating natural environmental changes under controlled experimental conditions using a natural phytoplankton community growing at near steady state in longer incubations (weeks, Hutchins et al. 2003, Hare et al. 2005, Hare et al. 2007). Our results suggest that the future trend of simultaneously elevated pCO_2 and temperature could have great effects on the North Atlantic phytoplankton community, especially on the calcification process of coccolithophores. This in turn could dramatically alter the vertical export ratio of inorganic carbon

to organic carbon (the so called 'rain ratio') and therefore influence the carbon cycle in the ocean.

MATERIALS AND METHODS

Experimental setup and sampling. The shipboard incubation was conducted between June 20 and July 14, 2005 on the R/V *Seward Johnson* II during the NASB 2005 cruise. The initial phytoplankton community was collected at 57.58°N, 15.32°W. A shipboard continuous culture incubation system (Ecostat) was used to carry out steady-state simulation experiments under defined projected pCO_2 and temperature conditions (Hutchins et al. 2003, Hare et al. 2005, Hare et al. 2007). Near-surface water (5 - 10 m) containing the intact North Atlantic bloom community was collected into a 50 L mixing carboy using a trace metal clean towed-intake surface water Teflon diaphragm pumping system (Bruland et al. 2005) and then was cleanly filtered through acid-washed 200 μm Nitex mesh to eliminate large zooplankton. The whole water was then dispensed into 24 acid washed clean 2.7 L clear polycarbonate bottles for incubation. Clean 50 L seawater medium reservoirs were filled with 0.2 μm in-line filtered seawater collected at the same time as the whole phytoplankton community, which was later used as medium for dilution during the continuous culture incubation. Due to low initial nutrient concentrations, modest levels of nitrate and phosphate (final concentrations 5 μmol L^{-1} and 0.31 μmol L^{-1}, respectively) were added into the medium and initial incubation bottles. The background silicate concentration was 0.7 μmol L^{-1}.

Two Ecostat systems were used to examine four treatments: (1) Ambient: 12°C and 39 Pa (380 ppm) CO_2; (2) High CO_2: 12°C and 76 Pa (750ppm) CO_2, with only pCO_2 increased; (3) High temperature: 16°C and 39 Pa CO_2, with only temperature increased; and (4) Greenhouse: 16°C and 76 Pa CO_2, with both temperature and pCO_2 increased simultaneously. To provide for robust statistical testing of treatment effects, six replicate bottles were used for each of the four treatments. The two incubation temperatures were controlled using a recirculating thermoregulation system consisting of a thermostat-controlled heat-exchange cooling system and in-line electric heaters. One of the Ecostats was maintained using this system at ambient SST (12°C), and the other was 4°C above ambient temperature, as has been predicted for high latitude ocean regimes by the year

2100 (Sarmiento et al. 1998, Sarmiento et al. 2004). Two pCO_2 levels were set by gentle bubbling (3 ml min^{-1}) of ambient air (39 Pa CO_2) and a HEPA-filtered commercially prepared air/CO_2 mixture (76 Pa CO_2). Ambient air was collected using an air pump with a HEPA-filtered intake near the ship's bow, to avoid the ship's exhaust gases. CO_2 equilibration was monitored throughout the experiment using both pH and dissolved inorganic carbon (DIC) measurements. The light levels of the incubators were adjusted using a combination of spectrally-corrected blue plastic (Hutchins et al. 1998) and neutral density shade screens, to provide an irradiance of 30% of the incident sea surface level (I_0) inside of the incubators.

The incubation was conducted in 'batch' growth mode for the first three days (T0, T1 and T2) without dilution of filtered seawater medium. The continuous incubation started on the forth day (T3) with a constant dilution rate of 0.5 d^{-1} and lasted until the final sampling day T14. The dilution rate of each bottle was adjusted individually by a separate peristaltic pump, with an inflow line going into the bottle from the top of the cap. The outflow tubing was connected at the shoulders of the bottles, and drained down through a port in the incubator side along outflow lines and finally into the enclosed outflow receiving bottles. All parts of the system were built of either Teflon or polycarbonate, and were rigorously acid cleaned prior to the experiment. The system was equipped with a compressed air-driven system to gently rotate the entire Plexiglas rack holding the Ecostat bottles inside the incubator through a 120" arc on a timed cycle (5 - 15 minutes) to ensure that the phytoplankton cells remained suspended in the bottles (Hutchins et al. 2003, Hare et al. 2005, Hare et al. 2007).

Daily sampling directly from the Ecostat bottles was limited to ∼10% of bottle volume to avoid significant perturbations of the nutrient input/biomass accumulation equilibrium. Due to this sampling volume limitation, daily samples were only taken for the measurements requiring relatively small volumes. These included chlorophyll *a* (Chl *a*), algal community structure (algal pigments by HPLC, flow cytometry and microscopic cell counts), microzooplankton communities (grazer counts), dissolved nutrients (nitrate, phosphate, and silicate), total and dissolved dimethylsulfoniopropionate ($DMSP_t$ and $DMSP_d$), DIC and pH, for which samples were taken directly from the bottles with a sampling syringe. Particulate organic carbon (POC), biogenic silica (BSi), particulate organic nitrogen (PON), and particulate organic phosphorus (POP) samples and water

for the microzooplankton grazing experiments (Rose et al. 2008, submitted) and other parameters were taken from the outflow collecting bottles at specific timepoints. On the final day (T14), all the samples were taken directly from the incubation bottles.

Seawater Carbonate System Measurements. Samples for DIC measurements were taken in 20 ml borosilicate vials, and fixed with 0.2 ml of a 5% $HgCl_2$ solution. The vials were sealed and stored at 4°C until analysis. DIC was measured in an acid sparging instrument (Walz & Friederich 1996). For analyses, 1.25 ml samples were injected into a sparging column where the CO_2 resulting from acid conversion of the DIC pool was quantified using a LiCor infrared analyzer with high precision flow control; replicate precision for seawater samples is about ± 0.06%. The pH was measured daily using a Model 220 pH meter (Denver Instrument Company, USA) immediately after sampling.

Phytoplankton Community and Biomass Analyses. Size fractionated Chl *a* samples were filtered at low vacuum onto 0.2 μm and 2 μm polycarbonate filters (Millipore), extracted in 90% acetone at -20°C in dark for 18 - 24 hours, and measured with a Turner 10-AU fluorometer (Welschmeyer 1994). Samples (400 - 1000 ml) for taxon-specific pigments were filtered onto GF/F filters (Whatman) under low vacuum at sea and immediately frozen in liquid nitrogen for later high performance liquid chromatography (HPLC) analysis in the laboratory. Photosynthetic pigments were separated on an automated Hewlett Packard 1050 HPLC system using a reverse-phase Waters Symmetry C-8 column and a solvent gradient containing methanol, aqueous pyridine, acetone, and acetonitrile (Zapata et al. 2000, DiTullio & Geesey 2002). A diode array detector recorded pigment spectra every 5 seconds over the wavelengths 350 to 600 nm and continuous chromatograms at 410, 440, and 455 nm. An HP 1046A fluorescence detector with excitation of 421 nm and emission at 666 nm (optimized for Chl *a*) was also used to identify and quantify Chl *a* and *c*. The system was calibrated by repeated injections of pigment standards isolated from a variety of unialgal cultures maintained in the laboratory (DiTullio & Geesey 2002).
Phytoplankton cell abundance was determined on preserved samples by both microscopy and flow cytometry. Samples of 50 ml or 100 ml for laboratory cell counts using microscopy were preserved by a final concentration of 1% gluteraldehyde and stored at 4°C in the dark until analysis. Autofluorescing cells were counted by epifluorescence microscopy with a 0.1 ml Palmer-Maloney cell-counting chamber. Before counting, samples were concentrated in the laboratory by low-pressure filtration using 0.2 μm polycarbonate filters and

then resuspended in one to two ml of the 0.2-μm filtrate. Due to imperfect preservation and uncertainties with identification of small algal cells (nano- and picoplankton), only larger microphytoplankton taxa were enumerated using microscopy. The smaller taxa were enumerated using flow cytometry. For flow cytometry, 2 ml of samples were preserved with 1% formaldehyde in cryovials, stored at -80°C in the dark, and then analyzed in the laboratory using a FACSCalibur flow cytometer to measure forward light scatter (related to particle size), and red fluorescence from chlorophyll (660 - 700 nm) (Olson et al. 1993).

Dissolved and Particulate Matter. Dissolved nutrient samples were taken by syringe directly from the incubation bottles. Samples were immediately 0.2 μm filtered and stored at -20°C. Samples were analyzed in the laboratory using a Flo-Solution IV analyzer (O/I Analytical, College Station, TX, USA). Total particulate carbon (TPC) and particulate organic nitrogen (PON) were measured by filtering 100 - 200 ml samples onto pre-combusted (450°C, 2 hours) 25 mm diameter Whatman GF/F glass fiber filters.
For particulate organic phosphate (POP), 100 - 200 ml samples were filtered onto pre-combusted (450°C, 2 h) Whatman GF/F glass fiber filters and rinsed with 2 ml of 0.17 mol L^{-1} Na_2SO_4. The filter was then put into 20 ml pre-combusted (450°C, overnight) borosilicate vials with 2 ml of 0.017 mol L^{-1} $MnSO_4$. The vial was covered with aluminum foil, dried at 95°C, and stored in a dessicator until analysis. For final analysis, vials were first combusted at 450°C for two hours, and 5 ml of 0.2 mol L^{-1} HCl was added to each vial after cooling. Vials were then tightly capped and heated at 80°C for 30 min to digest POP into inorganic phosphate. Digested POP samples were analyzed with the standard molybdate colorimetric method (Solorzano & Sharp 1980, Fu et al. 2005). BSi samples (100 - 200 ml) were filtered onto 0.6 μm 47 mm polycarbonate filters, dried at 60°C at sea, and then stored at room temperature until analysis. The samples were analyzed in the laboratory following the method of Brzezinski & Nelson (1995).
Small volumes of samples ($\leq$ 20 ml) were treated with 50% H_2SO_4 and preserved for the determination of total DMSP ($DMSP_t$). A second small volume of each sample ($\leq$ 20 ml) was then gravity filtered through a Whatman GF/F filter, and preserved with 50% H_2SO_4 for the determination of dissolved DMSP ($DMSP_d$, Kiene & Slezak 2006). All DMSP samples were stored at 4°C until the samples could be analyzed. Upon analysis, the sample were based-hydrolyzed to convert the DMSP to DMS, which was measured

using a cryogenic purge and trip system coupled to a Hewlett-Packard 5890 Series II gas chromatograph fitted with flame photometric detector (White 1982, DiTullio & Smith Jr. 1995). Particulate dimethysulfoniopropionate ($DMSP_p$) for each sample was calculated as the difference between $DMSP_t$ and $DMSP_d$.

Photosynthesis-irradiance curves. Photosynthesis -irradiance response (PE) curves were obtained by measuring primary productivity as a function of light by the ^{14}C uptake method on a radial photosynthetron similar to the design described by Babin et al. (1994). Approximately 750 ml of sample were inoculated with 2 mCi of $Na_2{}^{14}CO_3$ (Nordion) in a 2 liter acid-cleaned polycarbonate flask. Following gentle homogenization, fifty ml aliquots were then automatically dispensed into 36 acid-cleaned 60 ml polycarbonate culture flasks. The flasks were then incubated in triplicate on the photosynthetron at 12 different irradiances at the appropriate temperature (12°C or 16°C) for 2 hours. The irradiance source was an Osram metal halide bulb. Irradiance was measured before and after incubation using a Biospherical Instruments QSL-100 quantum meter. Time zero ^{14}C uptake rates were measured and subtracted from all experimental samples. After homogeneously mixing the two liter flask, triplicate samples (100 μl) for total ^{14}C activity (TA) were taken and added to a 7 ml scintillation vial containing 100 μl of a phenethylamine:MeOH (1:1) solution. Four ml of scintillation fluid (Ecolume) was added to the vials before determining the total radioactivity. After the two hour incubation, samples were immediately filtered and degassed overnight with 10% HCl, and then counted on a Beckman 6500 LSC corrected for quench using the external standards ratio. Photosynthetic rates were calculated from TA, final radioactivity and total DIC concentrations. The curves were fitted using the three parameter model of Platt & Gallegos (1980).

POC and PIC Production. POC and PIC (Particulate Inorganic Carbon) production rates were estimated with the micro-diffusion technique according to Paasche & Brubak (1994) with some modifications. Briefly, PIC was separated from the organic carbon by dissolving the calcite in H_3PO_4 and trapping the liberated $^{14}CO_2$ into a filter wetted with viscous organic base in a closed scintillation vial. The two fractions were radioassayed separately. Samples (75 ml) were incubated with 10 μCi $NaH^{14}CO_3$ added for 24 hours under the appropriate experimental conditions for each treatment inside the Ecostat incubators. Incubations were started after the daily sampling at noon (13:00 h). After incubation, the samples were filtered onto 25-mm diameter Whatman GF/F glass

fiber filters. The filters were placed into 20 ml scintillation vials with a plastic rim on the inside of the caps, and then 13 mm Gelman AE GF glass fiber filters (Pall Corporation, USA) were wetted with 200 μl of phenethylamine and attached onto the caps. One ml of 50% phosphoric acid was added to each vial, which was immediately air-tightened with the cap containing the basic filter. After incubation overnight on a shaker table, the two fractions were radio-assayed separately.

Microzooplankton Community Counts and Grazing experiments. Samples for microzooplankton enumeration were taken on T0, T4, T9 and T14 directly from the incubation bottles by the syringe sampling. Microzooplankton grazing experiments were conducted using the dilution technique of Landry & Hassett (1982) on T0 and T8. The details of these measurements are described and discussed in Rose et al. (2008, submitted).

Statistics. Pairwise comparison tests were conducted with one-way ANOVA (Kinnaer & Gray 1997). Outliers were removed using the Hampel identifier, as modified by Rousseeuw & van Zomeren (1990).

RESULTS

Phytoplankton biomass estimated as Chl *a* responded to elevated temperature and pCO_2 (Fig. 3a). Total Chl *a* concentration increased in all treatments and nearly doubled in the greenhouse treatment over the first three days of the experiment, during the batch incubation mode. As dilution began after day 3, Chl *a* concentrations started to decline and returned to near-initial levels by day 8. Thereafter, Chl *a* levels were relatively stable until the final day (T14) with an average concentration close to the initial value, indicating that the net growth rate (i.e. including grazing) of the total phytoplankton community was in balance with the dilution rate. During the final three days, Chl *a* biomass was highest in the greenhouse treatment relative to the other treatments (Fig. 3a, $p < 0.05$). During the incubation period, size-fractionated Chl *a* concentrations (Fig. 3b+c) and cell densities estimated by flow cytometry (Rose et al. 2008, submitted) suggested that nano- and microphytoplankton came to comprise the majority of the whole phytoplankton community biomass in all of the treatments. Size fractionated Chl *a* biomass associated with picophytoplankton declined during the course of the experiment to less than 0.2 μg L^{-1}, and there were no significant differences ($p > 0.05$) among treatments (Fig. 3c). The changes in micro- plus nano- phytoplankton Chl *a* (Fig. 3b) were similar to changes in

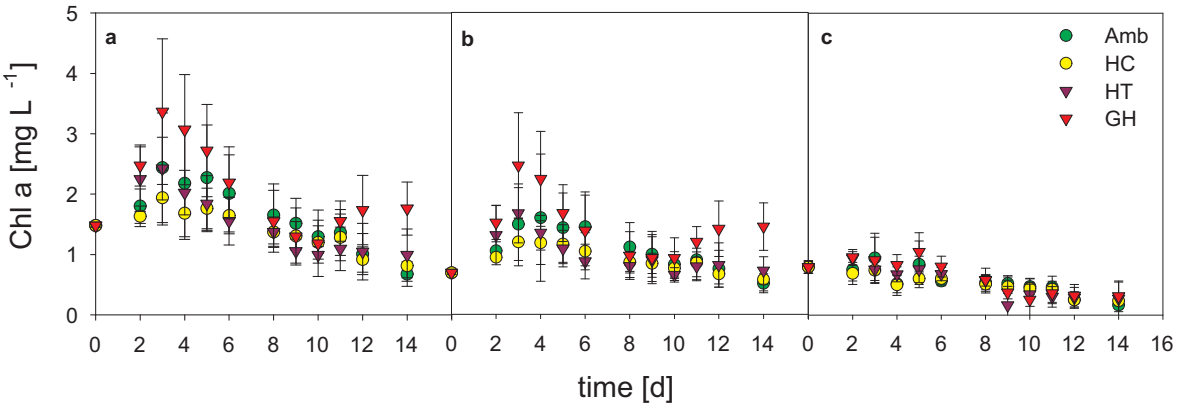

Figure 3: Chl *a* concentrations of (a) the total community, (b) microplankton and nanophytoplankton (> 2.0 μm), and (c) Picophytoplankton (0.2 μm - 2 μm) in the four incubation treatments. Amb = Ambient, HC = High CO_2, HT = High Temperature, GH = Greenhouse. Error bars represent standard deviations (n=6).

total Chl *a* (Fig. 3a). This large size fraction Chl *a* showed an initial increase during batch mode (especially in the greenhouse treatments), followed by a decline to near-initial values in all treatments, with the highest final levels in the greenhouse bottles ($p < 0.05$). Detailed descriptions of changes in Chl *a* biomass, community composition measured with flow-cytometry, and the microzooplankton community are presented in Rose et al. (2008, submitted).

Microscopy cell counts of microphytoplankton on the final day (T14) further demonstrated different effects of increased temperature and pCO_2 on different phytoplankton groups (Fig. 4). The three main phytoplankton groups observed during the incubation were diatoms, coccolithophores and chrysophytes. On the initial day (T0), haptophytes (mainly coccolithophores) were dominant in the phytoplankton community based on the phytoplankton pigment analyses (data not shown), although these were small species as microphytoplankton counts did not reveal a relatively higher abundance of coccolithophores than diatoms. In the microphytoplankton group, diatom abundance on the final sampling day (T14) increased dramatically in the high pCO_2 treatment relative to the other three treatments (> 3-fold, Fig. 4A). On the final day, coccolithophore abundance in the greenhouse treatment was significantly higher than in the other three treatments, and 5-fold higher than in the ambient treatment ($p < 0.05$, ANOVA, Fig. 4B). The cell

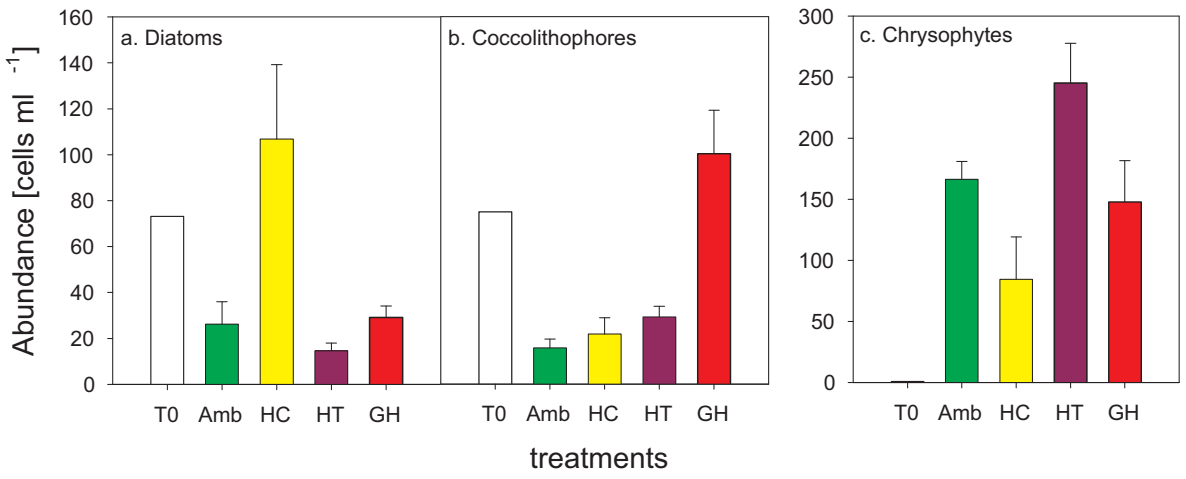

Figure 4: Microphytoplankton abundance on the initial day (T0) and in the four treatments on the final day (T14) by microscopy. (a) Diatoms, (b) Coccolithophores, and (c) Chrysophytes. Amb = Ambient, HC = High CO_2, HT = High Temperature, GH = Greenhouse. Error bars represent standard deviations (n=6).

abundance of chrysophytes was very low on T0 and increased in all of the four treatments during the time course of the incubation. Chrysophyte cell density on the final day was significantly higher ($p < 0.05$) at elevated temperature and lower ($p < 0.05$, ANOVA) at elevated pCO_2 within each temperature treatment (Fig. 4C). This effect of pCO_2 was not observed for diatoms or coccolithophores (Fig. 4A+B).

On the final day, 19-hexanoyloxyfucoxanthin (19-hex) concentration (indicative of haptophytes, in this case coccolithophores) was significantly higher ($p < 0.05$, ANOVA) in the greenhouse treatment than in other treatments, consistent with microscopy cell count estimates (Fig. 5). The final day 19-hex concentration was about 3-fold higher in the greenhouse than in the ambient treatment. In addition, the particulate DMSP ($DMSP_p$) to Chl *a* ratio on the final day was 2-fold higher in the greenhouse treatment than in the ambient treatment (Fig. 6), also indicative of high coccolithophore abundance. The $DMSP_p$ to Chl *a* ratio in each of the other three treatments was close to the initial ratio on T0.

Photosynthetic carbon fixation also responded to the experimental treatments (Fig. 7). Photosynthesis versus irradiance (PE) curves on the last day of the incubation demonstrated that maximum biomass-normalized photosynthetic rates (P^B_{max}) increased signif-

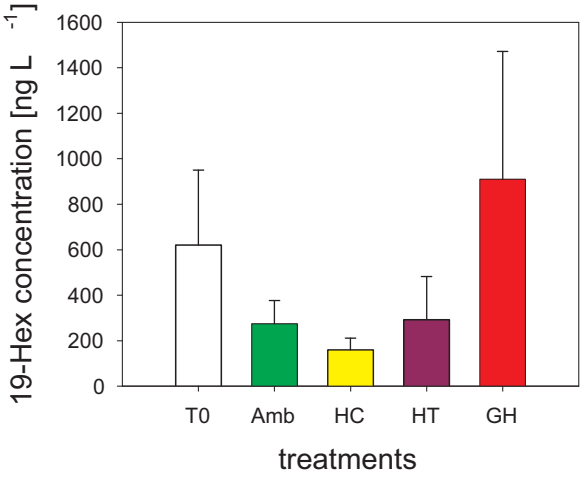

Figure 5: The concentration of the haptophyte pigment 19-hexanoyloxyfucoxanthin (19-Hex, primarily from coccolithophores) on the initial day, and in the four treatments on the final day. Amb = Ambient, HC = High CO_2, HT = High Temperature, GH = Greenhouse. Error bars represent standard deviations (n=6).

icantly from an average value of 8.2 to 12.8 ($p < 0.05$, ANOVA) in the two higher temperature treatments compared to the other two ambient temperature treatments (Fig. 7). Increased pCO_2 alone increased P^B_{max} slightly only at ambient temperature. The initial slope of the PE curves (α, with values of 0.057, 0.049, 0.077 and 0.103 g C h^{-1}(g Chl a)$^{-1}$(μE m^{-2} s^{-1})$^{-1}$ for ambient, high pCO_2, high temperature and greenhouse treatments respectively) was also increased significantly in the high temperature and greenhouse treatments ($p < 0.05$) with respect to ambient conditions.
The final day BSi to POC molar ratio in all the four treatments decreased compared to the T0 value. On the final day, the ratio was significantly lower ($p < 0.05$) in the high temperature and greenhouse treatments than in the two lower temperature treatments (Fig. 8). The molar ratio dropped by 30% (from ∼0.006 to 0.004) after the temperature was increased 4°C. However, within the same temperature conditions, there was no significant difference ($p > 0.05$) between either the ambient and high pCO_2 treatments or the high temperature and greenhouse treatments (Fig. 8). A similar trend was observed with BSi to PON molar ratios (data not shown).

On the final day, production rates of POC and PIC varied as a function of temperature and pCO_2 (Fig. 9), and both were increased compared to T0. POC production rate

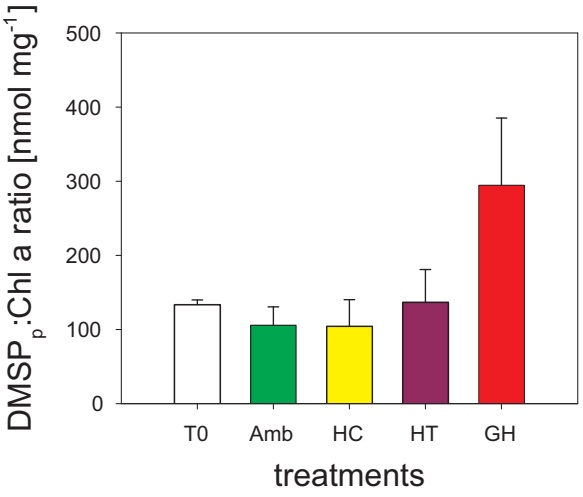

Figure 6: Particulate DMSP to Chl *a* ratio on the initial day, and in the four treatments on the final day. Error bars represent standard deviations (n=6).

was significantly higher in the two high temperature treatments (high temperature and greenhouse) ($p < 0.05$, Fig. 9a). The highest value of POC production was observed in the greenhouse treatment. However, despite the cell abundance of coccolithophores being highest in the greenhouse treatment (Fig. 4A), the PIC production was greatly reduced in these samples (Fig. 9a, $p < 0.05$). Increased pCO_2 alone (the high pCO_2 treatment) did not influence PIC productivity compared with the ambient treatment. Due to the decreased PIC productivity and increased POC productivity in the greenhouse treatment, the ratio of PIC production to POC production on the final sampling day was lowest in the greenhouse treatment (Fig. 9b, $p < 0.05$). There was no significant difference between the other treatments (Fig. 9b, $p > 0.05$).

For the measured cellular C:N:P ratios, transparent exopolymer particles (TEP) and molecular TEP to POC ratios, there was no statistically significant difference among all the four treatments (data not shown).

DISCUSSION

The North Atlantic spring bloom phytoplankton community responded significantly to the experimental treatments in this shipboard continuous incubation experiment. Treatment-specific community shifts were induced by both increased temperature and pCO_2 conditions, with the highest diatom abundance in the high CO_2 treatment, the highest

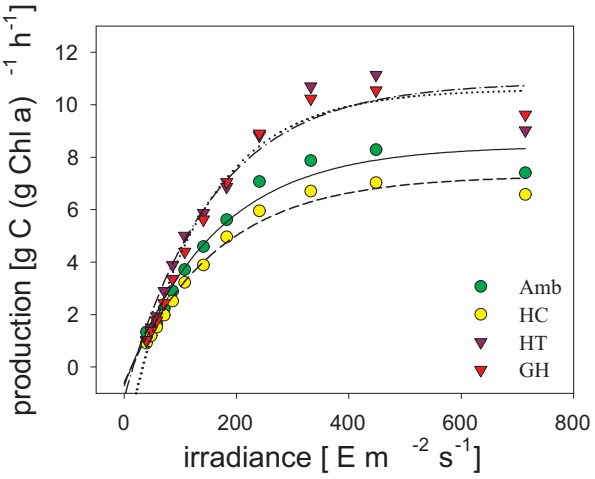

Figure 7: Photosynthesis versus irradiance curves of the four treatments on the final day (Triplicates combined). Amb = Ambient, HC = High CO_2, HT = High Temperature, GH = Greenhouse.

coccolithophore abundance in the greenhouse treatment, and the highest chrysophyte abundance at high temperature. Both photosynthetic parameters and POC production were greatly promoted by elevated temperature. In contrast, net calcification rate (PIC production) decreased significantly in the greenhouse treatment. Consequently, the potential marine rain ratio (as estimated by the ratio of PIC:POC production) was significantly lower when temperature and pCO_2 were elevated simultaneously, suggesting the possibility of a reduced export ratio of calcium carbonate relative to organic carbon in the future marine environment.

During the last few days of this incubation experiment, Chl *a* biomass was nearly constant in most treatments, indicating that community net growth was roughly balanced by the losses through the outflow. Thus, these experimental communities reached something approximating steady-state. However, this shipboard 'greenhouse ocean' simulation was a relatively short term (14 days) study compared to expected decadal-scale changes in global pCO_2 and temperature. Therefore, this experiment cannot replicate any possible long term adaptations and evolution of marine phytoplankton groups (Hutchins et al. 2003, Hare et al. 2007). As in all bottle incubations, grazing by large zooplankton and particle sinking were excluded, which are also important environmental factors.
Despite these qualifications, this type of shipboard incubation experiment is uniquely suited to provide us with a detailed perspective on how the current dominant phyto-

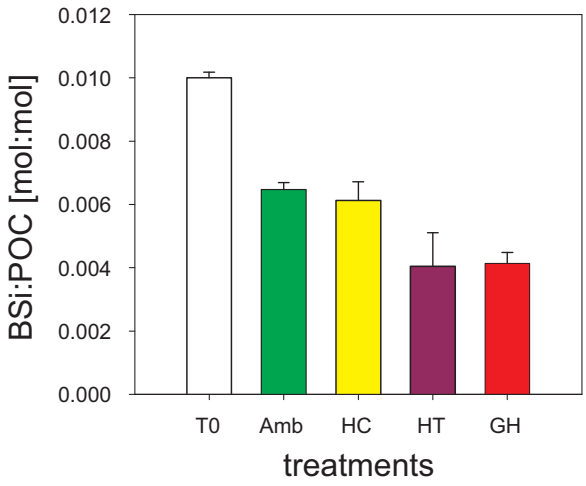

Figure 8: Cellular biogenic silica (BSi) to particulate organic carbon (POC) ratios on the initial day and in the four treatments on the final day. Amb = Ambient, HC = High CO_2, HT = High Temperature, GH = Greenhouse. Error bars represent standard deviations (n=6).

plankton groups in the North Atlantic spring bloom may respond to bottom-up control by projected future pCO_2 and temperature changes. Although too short to incorporate long-term biological adaptation processes, these experiments nevertheless offer valuable insights into which groups within the current phytoplankton community are already pre-adapted to benefit (or lose out) under expected future ocean conditions. In this way, manipulative experiments offer an additional tool to complement, enhance and extend the knowledge of ocean global change effects that is obtained from laboratory studies, time series stations, long term observations, and quantitative modeling efforts (Hare et al. 2007).

Our experimental results indicated that a 4°C temperature elevation induced higher algal carbon fixation rates by the North Atlantic phytoplankton community. POC production on the final sampling day was two-fold higher at the higher temperature than in the lower temperature treatments. Maximum photosynthetic rates and the slope of the light-limited portion of the PE curve also displayed similar trends. At the same time, the final Chl *a* biomass was highest in the greenhouse treatment. The dark reactions of photosynthesis are enzymatically-mediated and are thus known to be especially sensitive to temperature (Geider & Osborne 1992).

Previous studies have shown similar results, in that modestly increased temperature

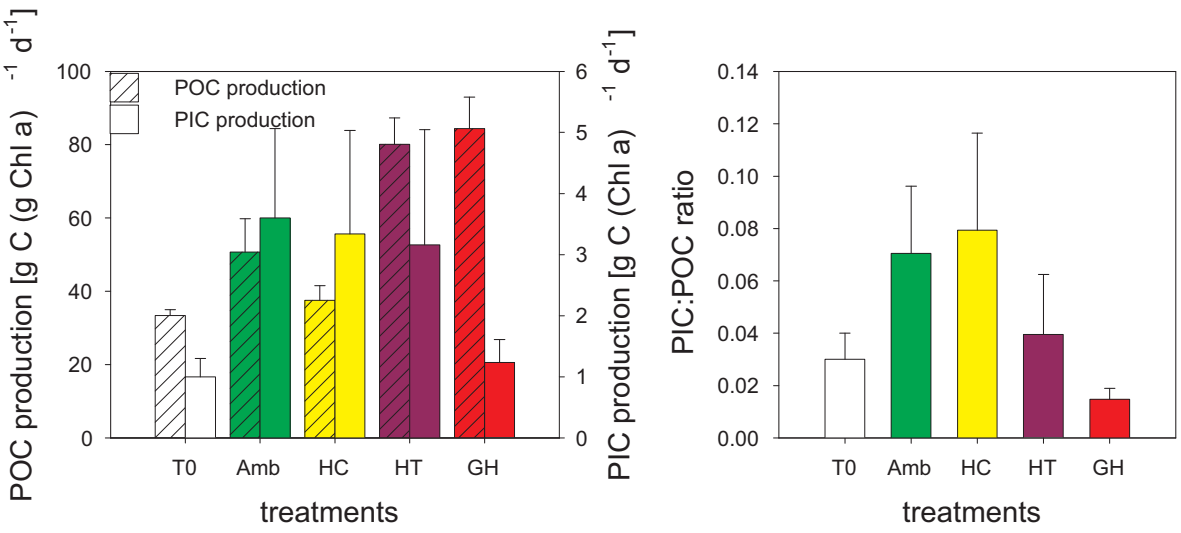

Figure 9: Production of (a) Particulate organic carbon (POC) and Particulate inorganic carbon (PIC) on the initial day, and in the four treatments on the final day, and (b) PIC to POC production ratios on the initial day and in the four treatments on the final day. Amb = Ambient, HC = High CO_2, HT = High Temperature, GH = Greenhouse. Error bars represent standard deviations (n=6).

greatly promoted phytoplankton photosynthetic parameters in laboratory cultures of marine cyanobacteria (Hutchins et al. 2007, Fu et al. 2007) and the coccolithophorid *Emiliania huxleyi* (Feng et al. 2008). Recent shipboard continuous incubation experiments similar to ours that used Bering Sea natural phytoplankton communities also found that, as long as photosynthesis is not limited by other factors such as nutrients or light, biomass-normalized maximum carbon fixation rates could potentially double with expected surface ocean warming trends over the next 100 years (Hare et al. 2007). This suggests possible accelerated carbon sequestration by marine phytoplankton from the atmospheric CO_2 reservoir, thus offering a negative feedback on atmospheric CO_2 and greenhouse warming. In contrast, increased pCO_2 alone had relatively little effect on community carbon fixation in our study, which is similar to the results from previous experimental studies in the tropical North Pacific (Tortell et al. 2002) and the Bering Sea (Hare et al. 2007). These increased carbon fixation rates were also accompanied by large phytoplankton community structure changes. By far the most striking shift was a greatly increased abundance of coccolithophores in the combined high temperature and pCO_2 environment (greenhouse treatment). The current sea water CO_2 concentration is far below the saturation level for

photosynthesis by marine coccolithophores (Riebesell et al. 2000, Rost et al. 2003). Our results found trends that were similar to those of these previous studies, indicating that coccolithophores will benefit from rising atmospheric pCO_2. Previous research has found that the photosynthetic carbon fixation rate of marine diatoms is close to saturation at the present day CO_2 level (Burkhardt et al. 1999; 2001, Rost et al. 2003). However, diatom abundance also increased after CO_2 enrichment according to our results, especially in the high pCO_2 treatment. Our results also suggested that chrysophytes have a lower CO_2 requirement compared to coccolithophores and diatoms, with lower abundance at higher pCO_2. In addition to possible direct effects of pCO_2 and temperature, the phytoplankton community structure changes we observed in this experiment are also undoubtedly a function of competition among the different groups under the four different experimental regimes.

This trend towards coccolithophorid dominance was dramatically enhanced when increased temperature and pCO_2 were increased simultaneously in the greenhouse treatment. A coupled influence of increased temperature and pCO_2 on coccolithophorid growth and physiology was also found in laboratory culture experiments with *E. huxleyi*, in which the photosynthetic carbon fixation was greatly promoted when both temperature and pCO_2 were increased (Feng et al. 2008). However, the physiological mechanisms driving these results are still unknown.

This change in algal community structure was also induced by the large shifts in microzooplankton species composition and abundance over the course of the experiment, as well as significant differences in grazing pressure by microzooplankton, as described in a companion paper (Rose et al. 2008, submitted). In general, throughout most of the experiment there was an active grazer community capable of consuming much of the daily primary production in all four treatments. However, towards the end of the experiment differences in top-down control by the microzooplankton community may have acted as a positive feedback for the growth of potentially unpalatable coccolithophore species in the greenhouse treatment (Rose et al. 2008, submitted). Such shifts in multiple trophic levels and their mutual interactions may thus be a feature of future changes in the North Atlantic spring bloom assemblage.

A previous semi-continuous incubation in the tropical Pacific found a diatom-dominated community developed after CO_2 enrichment to 750 ppm (Tortell et al. 2002), but this

study did not explore potential temperature interactions. Hare et al. (2007) reported that originally dominant large diatoms were replaced by smaller nanophytoplankton species after pCO_2 and temperature were both elevated in Bering Sea experiments. Our results also suggest that nanophytoplankton (coccolithophores) may be more favored in the future North Atlantic spring bloom if CO_2 concentration and sea-surface temperature continue to increase.

Nutrient and carbon biogeochemistry was influenced by the elevated temperature and pCO_2 as well. Following along with the decreased diatom abundance, the community BSi:POC ratio was significantly decreased by increased temperature, suggesting lower biogenic silica to particulate carbon export in the future warmer marine environment. Although coccolithophore abundance was by far the highest in the greenhouse treatment, the PIC production rate was nevertheless significantly reduced when pCO_2 and temperature were elevated simultaneously. Our results suggest that coccolithophores could be become even more dominant in the future North Atlantic spring bloom, while paradoxically calcification could decrease dramatically at the same time.

Riebesell et al. (2000), Zondervan et al. (2001), and Zondervan et al. (2002) found that CO_2 enrichment alone reduced the calcification of coccolithophores in laboratory incubations. Furthermore, an obvious malformation of coccoliths was observed at pCO_2 of 76- 89 Pa (750-880 ppm) in two coccolithophore species, *E. huxleyi* and *Gephyrocapsa oceanica* (Riebesell et al. 2000). In contrast, we found no effect of elevated pCO_2 alone on calcification of the North Atlantic natural coccolithophore community; only when pCO_2 was increased in concert with temperature was a significant reduction in the PIC:POC ratio observed. In keeping with this interactive effect of pCO_2 with other variables, a laboratory semi-continuous experiment using *E. huxleyi* also found significantly decreased cellular PIC content when pCO_2 was elevated to 76 Pa (750 ppm), but only when irradiance was saturating at the same time (Feng et al. 2008). Despite this striking illustration of the importance of other interacting variables, in general our results are consistent with predicted decreases in calcification in a high CO_2 ocean (Riebesell et al. 2000, Feely et al. 2004, Orr et al. 2005).

It has been hypothesized that bicarbonate (HCO_3^-) is the main or only carbon source for calcification, in contrast to the photosynthetic process, in which CO_2 is the main carbon source (Paasche 1964, Sikes et al. 1980, Rost & Riebesell 2004). The different response

of calcification and photosynthesis to changes in the marine carbonate system has been recognized to be the main cause of decreased PIC:POC (the rain ratio) with increased atmospheric CO_2 (Paasche 1964, Riebesell et al. 2000, Berry et al. 2002). Our on deck incubation was conducted under conditions of complete or near light saturation as determined by the light saturation value (E_k) from our P vs E experiments. Nevertheless decreased calcification was only observed in the greenhouse treatment when both pCO_2 and temperature were elevated simultaneously.

In addition to decreased calcification, we also observed large decreases in diatom abundance and the BSi to POC ratio under greenhouse conditions. These trends suggest possible significant future reductions in biomineral phases in general during the bloom. Since organic carbon export is thought to be heavily dependent on 'ballasting' by denser calcite and/or silica (Armstrong et al. 2002, Ziveri et al. 2007), such reductions in algal biomineralization could tend to reduce carbon export by the biological pump during the North Atlantic spring bloom.

We also recorded the highest $DMSP_p$ concentrations in the greenhouse treatment, accompanying the highest coccolithophore abundance. Many laboratory and field studies have found that marine haptophytes (including coccolithophores) and dinoflagellates generally have a higher cellular DMSP content than other groups (Keller et al. 1989, Malin & Stienke 2004). Since DMSP is the major precursor of DMS, coccolithophore (especially *E. huxleyi*) blooms are well known as high DMS production areas, sometimes with a large contribution from nanoflagellates or dinoflagellates (Archer et al. 2003, Steinke et al. 2002). Increases in DMS production under greenhouse conditions such as those we observed have the potential to increase cloud albedo, and thus be a negative feedback on global warming (Charlson et al. 1987). These results are consistent with the notion that this feedback may accelerate in the future, due to enhanced biomass-specific DMSP production resulting from changes in the North Atlantic spring bloom phytoplankton community in response to increased pCO_2 and temperature.

These experimental results provide new evidence indicating that further atmospheric CO_2 enrichment coupling with sea surface warming may have additive effects on the phytoplankton community of the North Atlantic spring bloom. We can speculate that under future global change scenarios, marine coccolithophores will be favored more than other groups such as diatoms. Biogeochemical consequences may include an increased organic

carbon fixation rate, significantly weakened calcification and silicification, and increased DMS production. Altogether, our experimental simulations of year 2100 pCO_2 and temperature suggest that future global changes may greatly influence algal productivity, community structure, and carbon, nutrient and sulfur biogeochemistry in the North Atlantic bloom.

Study II

Effects of inorganic and organic nitrogen and phosphorus additions on a summer phytoplankton community in the North Atlantic

INTRODUCTION

Different functional groups (according to Falkowski & Raven 1997) of phytoplankton have different nutrient demands and physiology (both macro- and micronutrients) and thus a competitive advantage under different nutrient regimes. Consequently, nutrient concentrations, ratios, and temporal variability all influence phytoplankton competition and therewith the phytoplankton assemblage (Hutchinson 1961, Tilman 1982, Gaedeke & Sommer 1986). The following discussion will be restricted to effects of macro-nutrients. Diatoms, for example, tend to dominate if silicic acid concentrations are high, whereas other functional groups dominate phytoplankton composition at low silicic acid concentrations (Egge & Aksnes 1992, Egge & Heimdal 1994). Within one functional group, competition also often depends on the availability of nutrients. Within the diatoms, for example, *Chaetoceros* spp. out-compete other diatom species when silicic acid concentrations are low (Harrison & Davis 1979), but nitrogen concentrations determine the diatom composition as long as silicic acid and phosphorus are replete (Niraula et al. 2007). Additionally, cell size plays an important role. Nitrate enrichment affects small- to medium-sized, chain building diatoms like *Chaetoceros* spp. faster than large ones (Carter et al. 2005), because the nitrate uptake of *Chaetoceros* (e.g. *Chaetoceros gracilis*) has much lower half saturation (K_s) values than the nitrate uptake of larger diatoms (Eppley et al. 1969) due to the higher surface to volume ratio of smaller cells. Also a high transcript induction of the gene encoding high-affinity nitrate transporters was detected by *Chaeto-*

ceros muelleri (Song & Ward 2007).

Phaeocystis cf. *pouchetii* is most competitive under low light and high nutrient conditions (Hegarty & Villareal 1998). *Emiliania huxleyi*, a coccolithophore, is a good competitor for orthophosphate, but does not grow well at low nitrate concentrations (Egge & Heimdal 1994). Competition between *E. huxleyi* and *Phaeocystis* spp. is influenced by nutrient concentrations and ratios as well as light and temperature. *E. huxleyi* seems to have only an advantage over *Phaeocystis* sp. if irradiance is above 20 μmol photons m^{-2} s^{-1} and temperature above 10°C (Egge & Heimdal 1994 and references therein).

But predictions as to which group out-competes others under specific conditions are difficult or even impossible to make. *Phaeocystis* spp. sometimes out-compete diatoms under high light, high silicic acid conditions and a low N:P ratio (Riegman et al. 1992), but at other times diatom species dominate under similar conditions (Egge & Aksnes 1992). Our inability to make reliable predictions of species composition and succession as a function of environmental factors may in part be due to a lack of understanding of all the important environmental parameters. The availability and utilization of organic nutrients, for example, is rarely considered.

Individual species of all major phytoplankton groups are able to utilize specific organic nutrients (Cembella et al. 1984, Palenik & Morel 1990, Antia et al. 1991, Ietswaart et al. 1994, Huang & Hong 1999, Peers et al. 2000, Berg et al. 2003, Yamaguchi et al. 2005, Shaked et al. 2006). Several phytoplankton species, including *E. huxleyi, Thalassiosira weissflogii, Phaeodactylum tricornutum, and Skeletonema costatum*, have been shown to take up and metabolize urea as an organic nitrogen source (Kristiansen 1983, Antia et al. 1991, Palenik & Henson 1997, Berg et al. 1997, Fan et al. 2003). As urea is an important organic nitrogen source in the open ocean (up to 0.5 μmol L^{-1}) and in estuaries (up to 8.9 μmol L^{-1}) (Antia et al. 1991 and references therein), it is likely to play a central role for the growth and competition of phytoplankton. Likewise, phosphomonoesters like glycerophosphate that constitute up to 39% of the total dissolved phosphorus (TDP) in coastal waters and up to 58% of the TDP in the open ocean (Benitez-Nelson 2000), should play an important role in determining phytoplankton assemblage. The utilization of glycerophosphate by different phytoplankton species has also been reported (Oh et al. 2002, Dyhrman & Palenik 2003, Shi et al. 2004).

Future ocean scenarios suggest that concentrations of nitrate and orthophosphate will

decline due to the rising surface temperature of the ocean and the resulting surface ocean stratification (Bopp et al. 2001). Stronger stratification will most likely impede the supply of new nutrients from deeper water layers and will prevent dissolved organic matter (DOM) produced by biological activity at the surface from mixing down at the same time. These changes will shift the inorganic to organic nutrient ratios in the ocean in favor of organic nutrients. In view of this change, the question of how the availability of organic nutrients influences the competition between phytoplankton groups and the phytoplankton community composition becomes important.

In this paper we investigate the effect of the addition of urea and glycerophosphate or nitrate and orthophosphate on the phytoplankton community composition of a natural phytoplankton assemblage of the North Atlantic. In spring, a diatom bloom characterizes the North Atlantic (Weeks et al. 1993), usually followed by a *Phaeocystis* spp. bloom (Wassmann et al. 2005 and references therein). In some years a coccolithophore bloom dominates in June and July after the diatom bloom (Tyrrell & Taylor 1996), but *Phaeocystis* spp. as well as dinoflagellates may also be present at this time (Weeks et al. 1993). As such, we entered this study hypothesizing that all functional groups would be present and could 'seed' the competition experiments.

MATERIALS AND METHODS

Experimental design. The impact of different nutrient sources on phytoplankton community composition was investigated on a natural plankton assemblage using a batch approach. Because we were strictly interested in comparing organic versus inorganic nitrogen and phosphorus sources, we did not assay nitrogen or phosphorus limitation. Seawater for the experiment was collected during the North Atlantic Spring Bloom (NASB) cruise in June/July 2005 southwest of Iceland at 64°N 25°W at the end of a large coccolithophore bloom. Water was pumped with a trace metal-clean Teflon pumping system (Hutchins et al. 2003, Bruland et al. 2005) from approximately 10 m depth and directly filtered through a 200 μm gauze to exclude large zooplankton. Fifteen 2.5-liter polycarbonate bottles were filled and triplicate bottles were spiked with nitrate and orthophosphate (hereafter NP treatment), nitrate and glycerophosphate (NGP treatment), urea and orthophosphate (UP treatment), and urea and glycerophosphate (UGP treatment). The additions consisted of 13 μmol N L^{-1} and 1 μmol P L^{-1}. Three control bottles (Cont)

were not spiked. All bottles were incubated for four days in a deck-board flow-through incubator. Initial (T_0) and final samples were taken and analyzed for dissolved nutrients, particulates, chlorophyll *a*, and cell counts (see below). During incubation, bottles received spectrally correct light adjusted to 40% of incident intensity (Hutchins et al. 1998) to avoid light damage.

Determination of dissolved nutrients. Water samples of 60 ml were filtered through precombusted glass fiber filters (Whatman GF/F) and stored at -24°C for the measurement of dissolved nutrients. Twenty ml of each sample was autoclaved with 2.4 ml sodium peroxidisulfate for 30 minutes to oxidize nitrogen and phosphorus compounds to nitrate and orthophosphate (Hansen & Koroleff 1999). Nitrate + nitrite (NO_x), ammonia (NH_4), orthophosphate (PO_4) and silicic acid (Si) concentrations were measured on the untreated samples and total dissolved nitrogen (TDN) and TDP concentrations were measured on the oxidized samples on an Alliance EVOLUTION^3 Auto-analyzer (Hansen & Koroleff 1999). Dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) were calculated as the difference of TDN and dissolved inorganic nitrogen (NO_x and NH_4) and the difference of TDP and PO_4, respectively. Because silicic acid samples were filtered through glass fiber filters which probably contaminated the samples, silicic acid concentrations were presumably overestimated.

Determination of particulate organic carbon, nitrogen, phosphorus, biogenic silica, and chlorophyll a concentration. Water samples (400 ml) from each bottle were filtered onto precombusted glass fiber filters (Whatman GF/F) and dried at 50°C. Filters were HCl fumed for 4 h in a dessicator, again dried in an oven at 60°C (Lorrain et al. 2003) and measured on a Carlos Erba Strumentazione Nitrogen Analyzer 1500 to determine the mass of particulate organic carbon (POC) and particulate organic nitrogen (PON).
For particulate organic phosphorus (POP) measurements, 200 ml samples were filtered onto precombusted glass fiber filters (Whatman GF/F) and rinsed with 2 ml of 0.17 molar Na_2SO_4. The filters were then put into 20 ml precombusted borosilicate scintillation vials with 2 ml of 17 mmolar $MnSO_4$ added. The vials were covered with aluminum foil, dried at 95°C, and stored in a dessicator until analysis. The vials were combusted at 450°C for 2 hours, and after cooling 5 ml of 0.2 molar HCl was added to each vial for final analysis. Vials were then tightly capped and heated at 80°C for 30 minutes to digest POP into inorganic phosphate. The digested POP samples were analyzed with the standard

molybdate colorimetric method (Solorzano & Sharp 1980, Fu et al. 2005).

Samples for biogenic silica (BSi) measurements were filtered (200 to 500 ml) onto polycarbonate filters (pore size 0.6 μm) and stored in plastic Petri dishes. Filters were dried at 60°C for 24 h and then stored at room temperature. Samples were analyzed for biogenic silica following the digestion of silica in hot NaOH for 45 min (Nelson et al. 1989).

Total chlorophyll *a* (Chl *a*) concentrations were determined by collecting samples on 0.22 μm nominal pore-size, 25 mm diameter polycarbonate filters (Osmonics). Following the non-acidification protocol of Welschmeyer (1994), samples were extracted in 90% acetone at 4°C for 24 hours and quantified using a solid-standard normalized 10-AU field fluorometer (Turner Designs).

Plankton community composition. Water samples of 400 ml were filtered onto cellulose nitrate filters (Sartorius, 47 mm, 0.45 μm pore-size) and dried at 50°C for coccolithophore cell counts. Pieces of the filters were sputter-coated with gold-palladium and imaged with a Philips XL-30 digital scanning field-emission electron microscope. Coccolithophores were counted from SEM images and coccolithophores per liter were calculated from counts, counting area, filter area and filtered volume. Samples for cell counts of other plankton (50 ml) were preserved with Lugol's solution and stored at 4°C in the dark. All three replicates of the NGP treatment and the control, but only two of the three replicates of the other treatments could be counted. Samples were concentrated with a sedimentation chamber (Hydrobios) and counted on an inverse microscope (Zeiss, IM 35) (Utermöhl 1958). At least 500 cells were counted and identified on a genera level using the taxonomic guide of Tomas (1996). Twenty representative individuals of the dominant genera and five representative individuals of the minor genera were sized (length and width) and the average volume calculated (Strathmann 1967) to determine the cell volume in μm^3 (CV) of the plankton. The cell volume was used to calculate the carbon content in pg C $cell^{-1}$ (CC) of the different genera (Strathmann 1967),

$$CC = CV \cdot 0.11 \tag{2}$$

The plasma volume rather than the cell volume is used to calculate CC for diatoms, because of their large vacuole. The length and width of the cells minus 0.5 μm respectively were used to calculate the vacuole volume (VV) (Strathmann 1967). The plasma volume in μm^3 (PV) was then calculated using the following equation:

$$PV = CV - (0.9 \cdot VV) \tag{3}$$

Phaeocystis spp. colonies disintegrate during fixation, thus only individual cells could be counted. The carbon content of the *Phaeocystis* spp. colonies, *i.e.* the carbon content of the cells plus the mucus matrix, was estimated according to Schoemann et al. (2005), assuming that cells were present in their colonial form based on optical observations during the experiment. Plankton carbon (PC) was calculated by adding the carbon content of the different genera.

Statistics. Means are calculated from the replicates of one treatment, but outliers were ignored in the calculation. Statistically changes in cell concentrations over time as well as differences in final cell concentrations between treatments were not significant. The low number of replicates combined with the large variability are the cause for this lack of statistical significance, even when differences were high. For example, the cell density of *Chaetoceros* spp. increased in the NGP treatment from 17,453 cells L^{-1} to 279,876 cells L^{-1} in one replicate and to 542,298 cells L^{-1} in the other replicate. This corresponds to an increase by a factor of 16 and 31, respectively. But a t-test finds no significant difference in cell density between the start and the end concentrations due to the high standard deviation. The analysis of variance (ANOVA) will, for the same reason also not detect differences between treatments. Non parametric statistics are not exercisable because of the low number of replicates. We thus evaluated our results according to the following criteria: Initial and end values were considered different if the initial value was outside the range of the standard deviation of the final value. Treatments were considered different if the standard deviations did not overlap.

RESULTS

Initial water. Seawater temperature was 10.1°C and salinity 35.19 psu during collection. Nutrient concentrations before addition were 2.6 ± 0.1 μmol L^{-1} NO_x, 0.82 ± 0.07 μmol L^{-1} NH_4, 0.19 ± 0.01 μmol L^{-1} PO_4, 0.45 ± 0.01 μmol L^{-1} Si, 3.9 ± 0.4 μmol L^{-1} DON, and 0.11 ± 0.01 μmol L^{-1} DOP.

Nutrients. Inorganic NO_x and PO_4 concentrations (Fig. 10) decreased in all treatments and the controls. The natural NO_x concentration decreased below the detection limit in the urea spiked treatments UP and UGP (hereafter U treatments) and the controls, whereas the nitrate concentration decreased by about 33 - 37% in the nitrate spiked treatments NP and NGP (N treatments) (Fig. 10a) . The natural orthophosphate concentration decreased also below the detection limit in the glycerophosphate spiked treatments

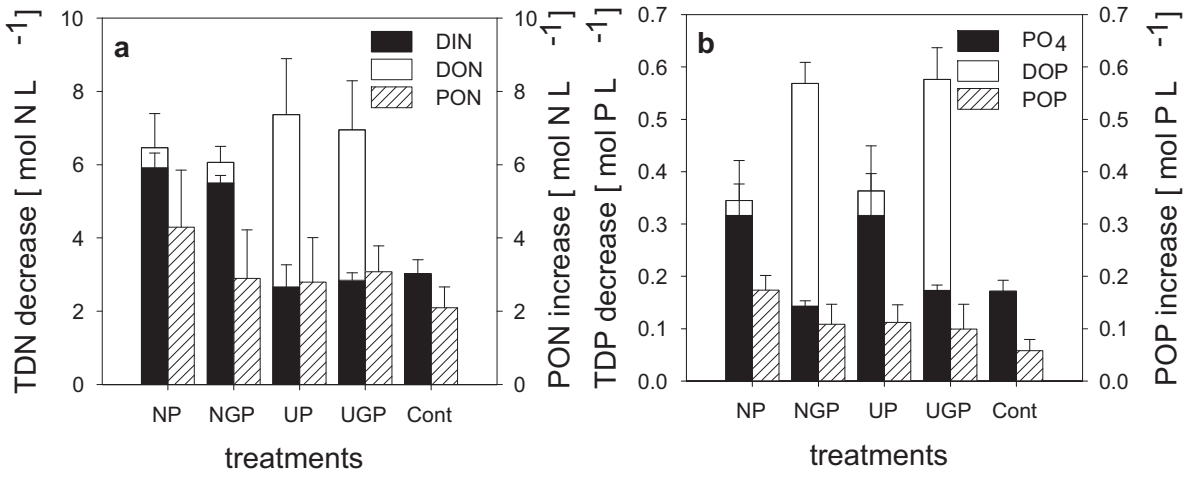

Figure 10: The decrease of dissolved nutrients and the according increase of particulate material for nitrogen (a) and phosphorus (b) during the experiment in the four treatments and the control. Columns show means ± standard deviations. TDN = total dissolved nitrogen, PON = particulate organic nitrogen, DIN = dissolved inorganic nitrogen (nitrate, nitrite and ammonium), DON = dissolved organic nitrogen, TDP = total dissolved phosphorus, POP = particulate organic phosphorus, PO_4 = orthophosphate, DOP = dissolved organic phosphorus, NP = nitrate/orthophosphate, NGP = nitrate/glycerophosphate, UP = urea/orthophosphate, UGP = urea/glycerophosphate, and Cont = control.

NGP and UGP (GP treatments) and the controls, and declined in the orthophosphate spiked treatments NP and UP (P treatments) by about 27% (Fig. 10b). The DON concentration decreased in the U treatments from 16.9 μmol L^{-1} to 12.2 and 12.8 μmol L^{-1}, respectively (Fig. 10a). The DOP concentration decreased in the GP treatments from 1.11 μmol L^{-1} to 0.69 and 0.71 μmol L^{-1}, respectively (Fig. 10b). Concentrations of naturally present DON and DOP did not change in the control.

The decrease of the TDN concentration was equal in all treatments ($\sim$6.7 μmol L^{-1}), but lower (3.0 μmol L^{-1}) in the control (Fig. 10a). The decrease of the TDP concentration was highest in the GP treatments (0.57 and 0.58 μmol L^{-1}, see Fig.10b). TDP declined by 0.34 and 0.36 μmol L^{-1} in the P treatments, and decreased by 0.17 μmol L^{-1} in the control (Fig. 10b).

Particulates and chlorophyll a. The POC, PON, POP, and BSi concentrations increased in all treatments and the control during the four days incubation (Tab. 1). Whereas POC concentration increased 4.5 fold, PON concentration tripled, and POP and BSi concentrations doubled. No differences were observed between treatments or control in

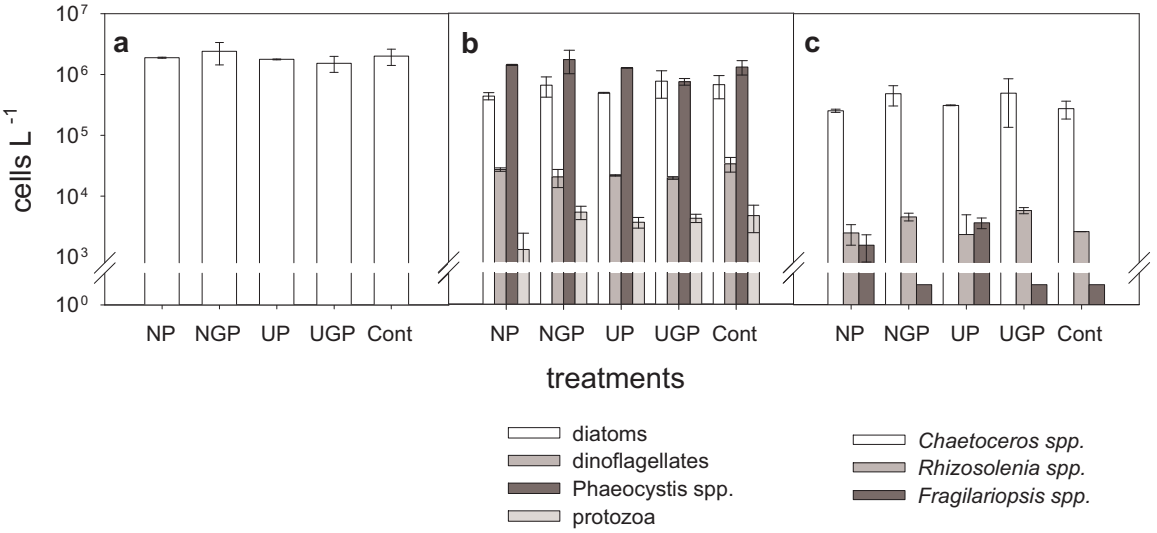

Figure 11: The total cell number (a), the cell numbers of selected diatom genera (b), and the cell numbers of different plankton groups (c) from the end of the experiment. Columns show means ± standard deviations. NP = nitrate/orthophosphate, NGP = nitrate/glycerophosphate, UP = urea/orthophosphate, UGP = urea/glycerophosphate, and Cont = control.

POC and BSi concentrations, but PON and POP concentrations exhibited a difference between the NP treatment and the control. Chl *a* concentration also increased, but less in the control than in the treatments. The ratios of POC:PON, POC:POP, POC:BSi, and POC:Chl *a* increased in all treatments and the control, but showed no differences between the treatments or the control (Tab. 1). The PON:POP ratios showed an increase by the factor of ∼1.5 in all treatments and the control, but did not differ between the treatments or the control (Tab. 1).

Plankton community. The initial plankton community consisted of several different diatom and dinoflagellate species, *Phaeocystis* spp., and a few protozoa. Coccolithophores were rare (<500 cells L^{-1}) (Tab. 2). Total cell abundance of the plankton increased 8 to 12 fold in all treatments and control during the 4 day incubation (Tab. 2) with no difference between the treatments and the control (Fig. 11a).

The total cell abundance of diatoms increased 5 to 8 fold during the experiment (Tab. 2) and did also not differ between the treatments and the control (Fig. 11b). This increase was overwhelmingly caused by the growth of *Chaetoceros* spp. (14 to 28 fold increase) (Tab. 2). Changes in concentrations of all other diatoms were comparably small. However,

some interesting patterns became visible. The mean cell density of the diatom *Fragilariopsis* spp. at the end of the experiment increased only in the P treatments, irrelevant of the nitrogen addition (Fig. 11c). Correlation coefficients were calculated for the increase of the carbon content of the different genera and the decrease of the different nutrient concentrations. The increase of the carbon content of *Fragilariopsis* spp. correlated positively to the decrease of orthophosphate concentration ($r = 0.90$, $n = 10$, $p < 0.001$). Positively correlated were also the increase in carbon content of *Chaetoceros* spp. as well as the carbon content of *Rhizosolenia* spp. to the decrease of DOP concentration ($r = 0.71$, $n = 12$, $p < 0.05$ and $r = 0.78$, $n = 10$, $p < 0.01$, respectively). The average increase in cell concentrations of *Chaetoceros* spp. and *Rhizosolenia* spp. were also higher in treatments with glycerophosphate (factor of 27 and 28, and 2.4 and 3.1, respectively) than in treatments with orthophosphate (factor of 14 and 18, and 1.3 and 1.2, respectively) (Fig. 11c), although these differences were obscured by the high variability between replicates.

The total cell abundance of dinoflagellates decreased 0.4 to 0.7 fold during the four days in all treatments (Tab. 2). The concentration of dinoflagellates did not change in the control, but standard deviation of the triplicate controls was extremely high (48% of the mean). The decrease in cell numbers of dinoflagellates was largely due to a decrease in concentrations of *Protoperidium* spp., *Dinophysis* spp., and *Prorocentrum* spp., which more than compensated the increase in the cell numbers of some unidentified dinoflagellates (Tab. 2). The mean cell abundance of the *Phaeocystis* spp. increased greatly (13 to 31 fold) in all treatments and the control (Tab. 2), but less in the UGP treatment compare to the other treatments and the control (Fig. 11b).

Overall, the abundance of protozoa did not change in the NP treatment, but increased 2 times in the all other treatments and the control (Tab. 2 and Fig. 11b) due to the increase of the cell density of naked ciliates in the NGP, UP, and UGP treatments and the control (Tab. 2).

The initial and final cell abundance of coccolithophores in all treatments and the control was too low to assess changes.

Total plankton carbon (PC) concentration (ignoring mucus from *Phaeocystis* colonies) did not change during the experiment in any treatment or the control (Tab. 1). Carbon content of *Phaeocystis* spp. cells increased moderately, but carbon concentration increased drastically when *Phaeocystis* colony mucus was added. Carbon content of the *Phaeocystis*

mucus was high and affected total PC values strongly. The PC concentration including the *Phaeocycstis* mucus (PC + mucus) increased in all treatments and the control from 64 to 147 - 229 μg C L^{-1} (Tab. 1). The POC:PC + mucus ratios increased in all treatments and the control, as well as the PC + mucus:PC and PC + mucus:BSi ratios (Tab. 1).

DISCUSSION

Nutrient uptake. Net concentrations of naturally present DON and DOP did not change in the control, suggesting that DON and DOP were not utilized, although production and loss could have been balanced. In the sampling region coccolithophores had formed a large bloom lasting over a month before the collection date of the water (Fig. 12). Coccolithophores, especially *E. huxleyi*, can utilize different DON and DOP compounds (Ietswaart et al. 1994, Palenik & Henson 1997, Waser et al. 1998, Dyhrman & Palenik 2003, Shaked et al. 2006) and a large bloom lasting over one month is expected to have stripped the water of most usable organic nutrients, providing a possible explanation for a lack of drawdown of naturally present DON and DOP.

NO_x was completely taken up in the U treatments and the control, but the POC:PON ratios (Fig. 13a) showed no differences between the treatments or the control, suggesting no growth limitation due to low nitrate availability. The decrease of DON in the U treatments reflects the utilization of the urea by these communities. Urea could have been utilized directly by phytoplankton (Kristiansen 1983, Antia et al. 1991, Fan et al. 2003), or by bacteria which may have remineralized urea to ammonium (Middelburg & Nieuwenhuize 2000, Jørgensen 2006, but see also Cho et al. 1996, Berg & Jørgensen 2006), to avoid nitrogen limitation. The low chlorophyll *a* concentration (Tab. 1) in the control compared to the treatments suggests an incipient nutrient limitation in the control (Cullen et al. 1992).

The GP treatments and the control showed a lack of orthophosphate due to the complete uptake of orthophosphate in these treatments, however, glycerophosphate was utilized in the GP treatments (indicated by the decrease in DOP). Although, due to high variability between replicates, no differences in POC:POP ratios (Fig. 14a) between the treatments or the control were observed, it may be noted that the P treatments show the lowest mean ratios. Together with the lower PC:POP ratios (Fig. 14b) on the P treatments compared to the control this suggests a better phosphorus supply in the P treatments. The PC:PON

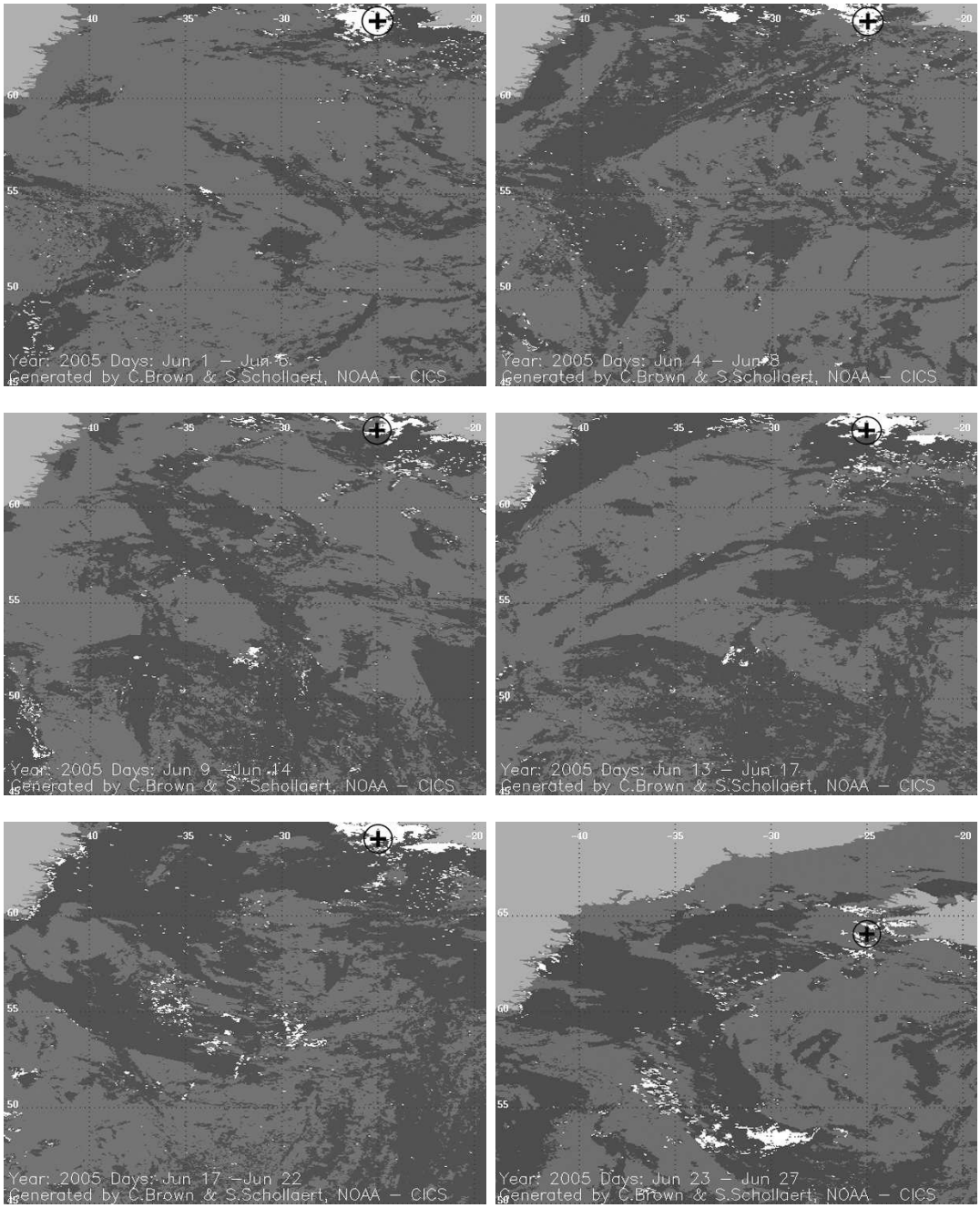

Figure 12: Satellite pictures of the sampling region from June 1^{st} to June 27^{th} 2005. In the northeast and northwest of the picture the landmasses of Iceland and Greenland are shown in light gray, respectively. Ocean surface is shown in dark gray and clouds in gray. White regions indicate the calcium carbonate of coccolithophore blooms. The black cross indicates the sampling station at June 29^{th} 2005.

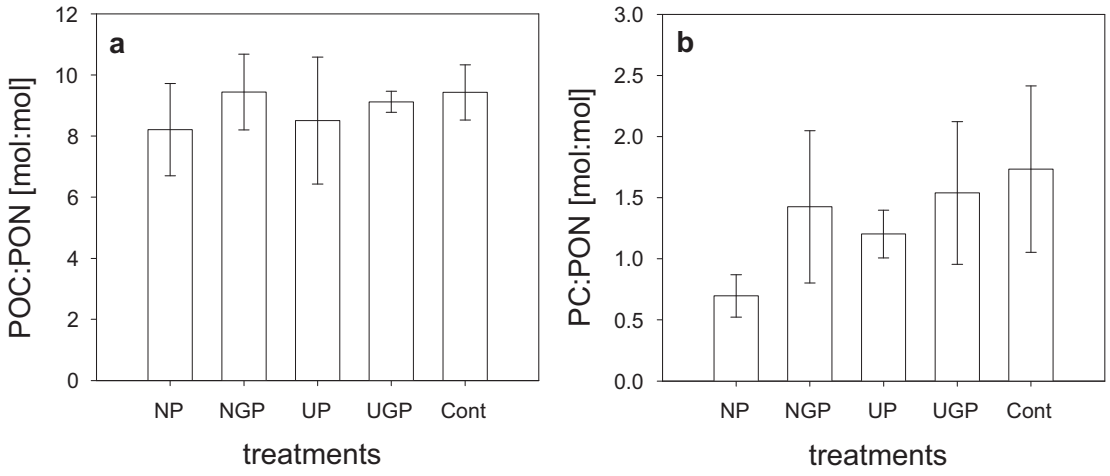

Figure 13: Particulate organic carbon to particulate organic nitrogen ratios (a) and plankton carbon to particulate organic nitrogen ratios (b) in the four treatments and the control at the end of the experiment. Columns show means ± standard deviations. POC = particulate organic carbon, PON = particulate organic nitrogen, PC = calculated plankton carbon, NP = nitrate/orthophosphate, NGP = nitrate/glycerophosphate, UP = urea/orthophosphate, UGP = urea/glycerophosphate, and Cont = control.

ratios (Fig. 13b) of the P treatments were lower than the ratios of the GP treatments and the control. Thus taken all these trends together, one could argue that better phosphorus supply in the P treatments enhanced nitrogen assimilation.

Urea and glycerophosphate were utilized heavily, but the build up of PON and POP can be explained by the loss of NO_x and PO_4 alone (Fig. 10). Total summed phytoplankton growth seems not to have been affected by the availability of organic nutrients. The budget of nitrogen and phosphorus is not balanced. In treatments spiked with organic nutrients (urea and/or glycerophosphate), the added organic nutrients were lost without appearing in a different pool of the budget. Possibly bacterial activity converted this material into substances not measurable with the standard procedure (Bronk et al. 2000).

The plankton community. Total cell abundance increased during the incubation (Tab. 2), consistent with the increased POC concentration during the experiment. But phytoplankton carbon is only one of several fractions of carbon making up the POC pool. Detrital matter contribute more than half to POC, e.g. in our experiment the PC + mucus provides 28 - 43% of POC. The increased POC:(PC + mucus) ratios (Tab. 1) in all treat-

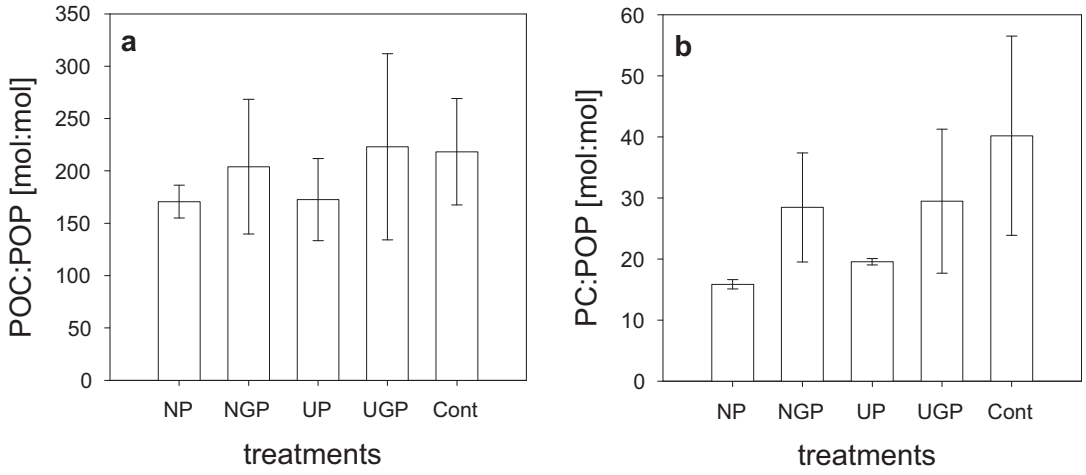

Figure 14: Particulate organic carbon to particulate organic phosphorus ratios (a) and plankton carbon to particulate organic phosphorus ratios (b) in the four treatments and the control at the end of the experiment. Columns show means ± standard deviations. POC = particulate organic carbon, POP = particulate organic phosphorus, PC = calculated plankton carbon, NP = nitrate/orthophosphate, NGP = nitrate/glycerophosphate, UP = urea/orthophosphate, UGP = urea/glycerophosphate, and Cont = control.

ments and the control indicate an increase of detritus during the experiment. In nature detritus is either eaten by zooplankton, remineralized by microbial activities, horizontally exported, or exported into deep water (Eppley et al. 1977). The lack of export in a bottle and reduced grazing (large zooplankton were filtered out) could be responsible for the increase in the amount of detritus in our experiment.

A shift was seen in the phytoplankton composition during the incubation, but this overall shift between different functional phytoplankton groups was the same in all treatments and the control (Fig. 11b) with the exception of *Phaeocystis* spp. (the cell concentration of this genus was lower in the UGP treatment). Cell concentrations of *Phaeocystis* spp. and the diatom *Chaetoceros* spp. rose during the incubation, with these species out-competing the other plankton in all treatments and the control. *Phaeocystis* spp. concentrations increased more than those of the diatoms and the increased PC + mucus concentrations (Tab. 1) during the 4 day incubation period indicate an increase of *Phaeocystis* spp. colonies. Also the increase of the PC + mucus:PC, and PC + mucus:BSi ratios (Tab. 1) argue for the increase of *Phaeocystis* mucus and therefore *Phaeocystis* colonies in all treat-

ments and the control.

The increase of *Phaeocystis* spp. and *Chaetoceros* spp. concentration in the control indicate other reasons than nitrogen and phosphorus additions for this increase of cell concentration. This increase could be due to bottle effects including changes in grazing pressure or light climate or due to the low silicic acid concentration found at the beginning of our experiment (silicic acid concentration of $\leq 0.45 \pm 0.01$ μmol L^{-1}). *Phaeocystis* spp. could get a competitive advantage under this conditions, because diatoms tend to dominate only under high silicic acid concentrations (Egge & Aksnes 1992, Egge & Heimdal 1994, Brown et al. 2003).

Within the diatoms, the small chain-forming genera *Chaetoceros* increased in all treatments and the control during the incubation. Nitrogen addition is thought to favor small- and medium-sized chain-forming diatoms (Carter et al. 2005), but the increase in the control of our experiment suggests more complex interactions. *Chaetoceros* sp. has been found to out-compete *Nitzschia delicatissima*, *Leptocylindrus danicus* and other diatom species at low silicic acid concentrations (Harrison & Davis 1979), which reflects the findings of our experiment. The dominance of *Chaetoceros* spp. in our experiment after the incubation can possibly be explained by a low threshold concentration of silicic acid. But the Michaelis constant (K_s) for silicic acid is rarely known and can differ for two species of the same genus, e.g. *Thalassiosira pseudonana* and *T. weissflogii* have very different K_s values of 1.4 and 4.7 μmol Si L^{-1}, respectively (Del Amo & Brzezinski 1999). The increase of specific genera in all treatments and the control during the incubation period could be a bottle effect or caused by the low silicic acid concentration, but differences between the treatments or the control at the end of the experiment indicate a different utilization of the added nutrients. The cell concentration of the total plankton did not differ between the treatments or the control, but individual genera showed differences between the treatments. Cell concentration of the diatom *Fragilariopsis* spp. was higher in the P treatments (Fig. 11c), indicating a need of inorganic phosphorus of this genus. The correlation between the biomass increase of *Fragilariopsis* spp. and the decrease of the orthophosphate concentration in all treatments and the control confirm this assumption. Nothing is known about the ability of *Fragilariopsis* spp. to utilize organic phosphorus, but the apparent need of inorganic phosphorus in *Fragilariopsis* spp. suggests a disability of this genus to utilize DOP. Further experiments are needed to study this

possibility. The diatoms *Rhizosolenia* spp. and *Chaetoceros* spp. had a higher cell density in the GP treatments (Fig. 11c) and the biomass increase of these genera correlate with the decrease in DOP concentration. Several species of *Rhizosolenia* and *Chaetoceros* are known to generate alkaline phosphatase (Ou et al. 2006), also the ability of *Chaetoceros ceratosporus* to utilize phosphate diester is known (Yamaguchi et al. 2005). The higher cell concentrations of *Rhizosolenia* and *Chaetoceros* in the GP treatments and the correlation with DOP concentration suggest a biological relevance of DOP for these genera. The opportunity of *Chaetoceros* and *Rhizosolenia* to utilize organic phosphorus compounds indicates a competitive advantage of these genera compared to *Fragilariopsis* when DOP is available. In the GP treatments the cell density of *Rhizosolenia* spp. increased more than that of *Fragilariopsis* spp., but in P treatments and the control the opposite effect was observed, supporting the idea that *Rhizosolenia* spp. has an competitive advantage under high DOP concentrations.

Cell numbers of naked ciliates in the NP treatment were lower than in any of the other treatments. This must be due to an indirect effect, e.g. possibly bacterial growth was enhanced in the treatments spiked with organic nutrients (with urea, glycerophosphate or both), providing higher prey concentrations. Indirect effects, that could cause such a response, were not investigated in this experiment, but these results indicate that the food web structure, at least the microbial loop may change as a result of the availability of inorganic or organic nutrients.

Overall the impact of organic nutrients on functional groups were small during our experiment. Possibly the availability of organic nutrients would have a larger impact on functional groups in the total absence of nitrate and orthophosphate. Nonetheless some diatom genera were influenced by the ratio of inorganic to organic nutrients. *Chaetoceros* spp. and *Rhizosolenia* spp. seemed to utilize organic phosphorus components, whereas *Fragilariopsis* spp. required orthophosphate to grow. Our data also suggests that the ratio of organic to inorganic macro-nutrients may impact the microbial loop. The underlying mechanisms of this change in the food web structure needs to be evaluated in future experiments.

Table 1: The particulate organic carbon (POC), nitrogen (PON), phosphorus (POP), biogenic silica (BSi), and chlorophyll *a* (Chl *a*) concentrations and the ratios of the particulates at the beginning and the end of the experiment in the different treatments and the control. Ratios in mol:mol, only ratios with Chl *a* are in g:g. Means of the replicates ± standard deviations are shown. POC = particulate organic carbon, PON = particulate organic nitrogen, POP = particulate organic phosphorus, BSi = biogenic silica, Chl *a* = chlorophyll *a*, PC = plankton carbon, PC + mucus = plankton carbon plus calculated *Phaeocystis* mucus, T_0 = start of the experiment, NP = nitrate/orthophosphate, NGP = nitrate/glycerophosphate, UP = urea/orthophosphate, UGP = urea/glycerophosphate, and Cont = control.

	POC	PON	POP	BSi	PC	PC + mucus	Chl *a*
	μg C L^{-1}	μg N L^{-1}	μg P L^{-1}	μg Si L^{-1}	μg C L^{-1}	μg C L^{-1}	μg L^{-1}
T_0	118 ± 13	24 ± 7	3.8 ± 0.4	7.8 ± 0.2	64 ± 0	64 ± 0	1.17 ± 0.03
NP	612 ± 112	89 ± 22	9.2 ± 0.9	14.1 ± 0.8	53 ± 7	184 ± 7	3.94 ± 0.11
NGP	481 ± 82	69 ± 19	7.2 ± 1.2	13.4 ± 0.9	70 ± 26	229 ± 26	3.55 ± 0.14
UP	549 ± 90	68 ± 17	7.3 ± 1.0	14.0 ± 0.8	53 ± 4	171 ± 4	3.49 ± 0.17
UGP	564 ± 89	72 ± 10	6.9 ± 1.5	14.0 ± 0.8	76 ± 23	147 ± 25	3.96 ± 0.27
Cont	468 ± 56	58 ± 8	5.6 ± 0.7	14.2 ± 0.7	76 ± 26	199 ± 26	2.51 ± 0.10

	POC:PON	POC:POP	PON:POP	POC:BSi	POC:Chl *a*	POC:PC + mucus	PC + mucus:PC	PC + mucus:BSi
T_0	5.4 ± 0.0	82 ± 0	15.0 ± 0.0	35 ± 0	101 ± 0	1.8 ± 0.0	1.0 ± 0.0	19 ± 0
NP	8.2 ± 1.5	171 ± 16	21.3 ± 4.9	103 ± 25	155 ± 28	3.6 ± 0.3	3.5 ± 0.3	31 ± 3
NGP	9.4 ± 1.2	204 ± 62	22.3 ± 8.8	96 ± 11	154 ± 24	2.4 ± 0.5	3.5 ± 1.0	40 ± 6
UP	8.5 ± 2.1	173 ± 39	21.4 ± 8.8	80 ± 11	138 ± 26	2.8 ± 0.8	3.2 ± 0.2	29 ± 3
UGP	9.1 ± 0.3	223 ± 89	24.3 ± 9.0	94 ± 12	144 ± 34	3.6 ± 0.6	2.0 ± 0.3	25 ± 6
Cont	9.4 ± 0.9	218 ± 51	23.2 ± 5.5	78 ± 13	187 ± 29	2.4 ± 0.2	2.8 ± 0.8	33 ± 5

Table 2: Cell numbers in the different treatments. All concentrations in 10^3 cells L^{-1}. Means of the replicates ± standard deviations are shown. T_0 = start of the experiment, NP = nitrate/orthophosphate, NGP = nitrate/glycerophosphate, UP = urea/orthophosphate, UGP = urea/glycerophosphate, and Cont = control.

Genus	t_0	NP	NGP	UP	UGP	Cont
Pseudo-Nitzschia spp.	27.4	31.3 ± 4.0	29.3 ± 15.7	34.6 ± 12.4	64.0 ± 1.1	56.1 ± 19.4
Nitzschia spp.	3.1	4.1 ± 1.5	1.5 ± 0.4	2.1 ± 1.5	2.5 ± 1.7	9.6 ± 5.4
Asteriellopsis spp.	1.2	3.4 ± 1.1	10.9 ± 1.3	8.3 ± 5.8	9.8 ± 10.4	16.9 ± 16.8
Fragilariopsis spp.	<0.5	1.6 ± 0.7	<0.5 ± 0.0	3.6 ± 0.7	<0.5 ± 0.0	<0.5 ± 0.0
Cylindrotheca spp.	<0.5	1.8 ± 1.1	<0.5 ± 0.0	<0.5 ± 0.0	2.6 ± 0.1	6.3 ± 6.1
Chaetoceros spp.	17.5	252.2 ± 16.1	478.1 ± 175.2	308.5 ± 5.1	490.0 ± 356.2	273.3 ± 88.6
Thalassiosira spp.	8.1	9.3 ± 2.9	8.9 ± 3.2	4.7 ± 0.7	12.2 ± 0.4	13.3 ± 4.2
Rhizosolenia spp.	1.9	2.5 ± 0.9	4.5 ± 0.7	2.3 ± 2.6	5.8 ± 0.7	2.6 ± 0.0
Guinardia spp.	29.3	113.2 ± 16.8	107.8 ± 59.8	102.8 ± 9.5	155.4 ± 14.7	261.0 ± 176.7
Leptocylindrus spp.	0.6	<0.5 ± 0.0	3.7 ± 1.8	2.6 ± 3.7	1.9 ± 2.6	<0.5 ± 0.0
unidentified diatoms	2.5	19.1 ± 11.0	13.1 ± 5.5	16.0 ± 16.8	15.0 ± 16.0	32.0 ± 16.3
total diatoms	**91.6**	**432.6 ± 59.0**	**653.1 ± 239.4**	**485.4 ± 7.7**	**759.2 ± 361.4**	**664.2 ± 273.4**
total coccolithophores	**<0.5**	**<0.5 ± <0.5**	**<0.5 ± <0.5**	**<0.5 ± <0.5**	**<0.5 ± <0.5**	**<0.5 ± <0.5**
Protoperidium spp.	28.1	10.6 ± 4.0	7.2 ± 0.9	5.7 ± 2.2	8.4 ± 3.0	8.6 ± 6.2
Dinophysis spp.	3.1	<0.5 ± 0.4	<0.5 ± 0.0	<0.5 ± 0.0	<0.5 ± 0.0	<0.5 ± 0.0
Prorocentrum spp.	10.6	3.6 ± 2.2	2.1 ± 2.4	1.0 ± 0.0	<0.5 ± 0.0	6.7 ± 2.2
Ceratium spp.	0.6	<0.5 ± 0.4	<0.5 ± <0.5	<0.5 ± <0.5	<0.5 ± 0.0	<0.5 ± <0.5
unidentified dinoflagellates	3.1	11.9 ± 3.7	11.4 ± 3.4	14.5 ± 1.5	10.2 ± 3.2	19.7 ± 4.8
total dinoflagellates	**45.5**	**26.6 ± 1.8**	**20.2 ± 6.7**	**21.3 ± 0.6**	**19.3 ± 0.9**	**33.2 ± 9.1**
***Phaeocycstis* spp.**	**55.7**	**1406.0 ± 27.5**	**1720.6 ± 718.0**	**1257.5 ± 13.5**	**743.3 ± 91.7**	**1299.4 ± 344.3**
tintinnids	<0.5	<0.5 ± 0.0	<0.5 ± 0.0	<0.5 ± 0.0	<0.5 ± 0.0	<0.5 ± 0.0
other ciliates	1.9	1.3 ± 1.1	4.7 ± 0.4	3.6 ± 0.7	4.2 ± 0.6	4.2 ± 2.9
total protozoa	**1.9**	**1.3 ± 1.1**	**5.3 ± 1.3**	**3.6 ± 0.7**	**4.2 ± 0.7**	**4.7 ± 2.2**
unidentified flagellates	<0.5	7.2 ± 7.3	6.3 ± 0.1	6.2 ± 0.0	8.9 ± 5.3	13.3 ± 11.5
total cell number	**194.7**	**1879.4 ± 33.7**	**2401.8 ± 969.5**	**1770.9 ± 24.3**	**1534.9 ± 447.5**	**2008.8 ± 601.0**

Study III

Effects of urea on calcification of the coccolithophore *Coccolithus pelagicus* (Haptophyceae)

INTRODUCTION

One consequence of a rising mean global temperature is an increase in surface ocean stratification and a concomitant decrease in the input of inorganic nutrients from deeper layers (Bopp et al. 2001). This reduced availability of nitrate and other macro-nutrients might impact physiological processes as well as the ecological success of major phytoplankton taxa, such as diatoms and coccolithophores. *Emiliania huxleyi*, a species of the latter group, displays reduced photosynthesis, elevated calcite content, and consequently a higher cellular ratio of inorganic to organic carbon under nitrate limitation (Paasche 1998, Berry et al. 2002). The latter might in turn alter the rain ratio, *i.e.* the ratio of inorganic to organic carbon of sinking matter in the oceans, a central parameter in the global carbon cycle (for discussion see Rost & Riebesell 2004).

However, nitrate is not the sole sources of nitrogen in the ocean. The largest fraction of the total dissolved nitrogen (TDN) consists of organic components (Antia et al. 1991). Dissolved organic nitrogen (DON) contributes up to 89% to the TDN of the open ocean surface waters and reaches concentrations up to 7.4 μmol N L^{-1} in the North Atlantic (Berman & Bronk 2003 and references therein).

In view of the stratification-induced decline in nitrate availability the question whether also organic nitrogen can be utilized becomes more important. Previous studies have shown the potential to utilize organic nitrogen in various taxa of phytoplankton (e.g. Antia et al. 1991, Ietswaart et al. 1994, Peers et al. 2000). Urea is an important organic nitrogen source in the open ocean (up to 0.5 μmol L^{-1}) and in estuary systems (up to 8.9 μmol L^{-1}) (Antia et al. 1991 and references therein). Several phytoplankton species

were shown to take up and metabolize urea (Antia et al. 1991, Palenik & Henson 1997, Fan et al. 2003).

Although it was shown that *E. huxleyi* utilizes urea (Palenik & Henson 1997) it is still an open question whether the ability to grow on urea is a typical feature of coccolithophores. Since *E. huxleyi* is an atypical coccolithophore, inter alia in phylogenetic terms (Sáez et al. 2003), it is desirable to gain information about other coccolithophore species. In contrast to *E. huxleyi*, *Coccolithus pelagicus* is a typical coccolithophore with regard to phylogeny (Sáez et al. 2003). Additionally, *C. pelagicus* is the prime calcium carbonate producer in the northern North Atlantic (Young & Ziveri 2000, Ziveri et al. 2004) and thus ideally suited to complement the information, which research on *E. huxleyi* provides.

The present study investigates the utilization of the organic nutrient urea by *C. pelagicus* and the effects of this utilization on calcification rate and coccolith morphology of this species.

MATERIALS AND METHODS

Experimental design. A non-axenic monospecific culture of *C. pelagicus* (strain AC400, ALGOBANK www.nhm.ac.uk/hosted_sites/ina/CODENET/caencultures.htm) was grown in dilute batch cultures to study the influence of the nitrogen sources nitrate and urea on growth and calcification rate. Cultures were grown at a light intensity of 270 μmol photons m^{-2} s^{-1} in a 16:8 light:dark cycle and a temperature of 17°C.

Because previous tests have shown that *C. pelagicus* grew malformed in artificial seawater, natural pretreated North Sea water was used. In order to remove nitrate, a 25 L batch of water was 0.22 μm filtered (Durapore Hydrophilic Cartrige, Millipore) and autoclaved (121°C for 40 min), vitamins and trace metals were added according to f/4 (Guillard & Ryther 1962) and inoculated with *E. huxleyi* (strain PML B92/11), which was grown for four days. Dissolved inorganic nitrogen was measured to assure the complete uptake of inorganic nitrogen. Cells and particles were then removed by 0.2 μm (PALL Life Sciences Capsule AcroPak^{TM} 500) filtration. Because *E. huxleyi* changed the carbonate system by calcification and photosynthesis, total alkalinity (TA) and dissolved inorganic carbon (DIC) was measured before and after the growth of *E. huxleyi* and the change in DIC and TA was compensated by adding sodium bicarbonate (480 μmol L^{-1}). Additionally the pH was shifted to around 8.2 with 0.74 ml of 0.5 molar HCl. Then the media was spiked with

vitamins (f/4 concentrations), trace metals (f/4 concentrations), selenium (5 μmol L^{-1}) and nutrients in concentrations of 100 μmol N L^{-1} and 6.3 μmol P L^{-1}.
Before the experiment *C. pelagicus* was grown for 8 generations in the respective media of the two treatments to acclimate the culture to the different nutrient sources. For the experiment *C. pelagicus* was inoculated into 2 l Nalgene polycarbonate bottles in triplicate with an initial cell concentration of 600 cells ml^{-1}. The experiment was stopped when a cell concentration of ∼10,000 cells ml^{-1} was reached, which should ensure that changes of the carbonate system were insignificant during the experiment.
Samples for analysis of nutrients, TA and DIC were taken at the beginning and the end of the experiment. Samples for the determination of particulate organic carbon (POC), particulate inorganic carbon (PIC), particulate organic nitrogen (PON), and scanning electron microscopy (SEM) were taken at the end of the experiment.

Determination of nutrients and the carbonate system. Samples for dissolved inorganic and organic nutrients were filtered through a precombusted glass fiber filter (Whatman GF/F), and nitrite + nitrate (NO_x), ammonia (NH_4), and orthophosphate (PO_4) were measured on an Alliance EVOLUTION^3 Auto-analyzer (Hansen & Koroleff 1999). The filtered water samples for TDN were autoclaved with potassium peroxidisulfate at a temperature of 121°C for 30 minutes (Grasshoff et al. 1999). In this process all dissolved nitrogen was oxidized to nitrate and also measured with an Alliance EVOLUTION^3 Auto-analyzer. DON was calculated by subtracting the dissolved inorganic nitrogen from TDN.
TA samples were filtered (Whatman GF/F), stored at 0°C in the dark for 17 to 36 days and calculated from linear Gran plots (Gran 1952) after duplicate potentiometric titration (Bradshaw et al. 1981, Brewer et al. 1986). DIC samples were also filtered (0.2 μm, Sartorius), stored at 0°C in the dark and measured in triplicate using a Shimadzu TOC 5050A analyzer. The carbonate system was calculated from temperature, a salinity of 32, and the concentrations of DIC, TA, and orthophosphate, using the program CO2sys written by Lewis & Wallace (1998). The equilibrium constants of Mehrbach et al. (1973) as refitted by Dickson & Millero (1987) and the NBS scale for the pH were used.
Growth rate determination. Cell concentrations were determined every one to three days with an inverted light microscope (Zeiss, Axiovert 135) using a Sedgewick Rafter Cell from PYSER–SGI. Growth rate μ was calculated according to Eq. 4 where N_0 is the cell concentration at the beginning and N_{final} the cell concentration at the end of the

experiment, Δt are the days of the experiment.

$$\mu = \frac{\ln N_{final} - \ln N_0}{\Delta t} \quad (4)$$

Because cells in the urea treatment reached the stationary phase before final sampling, growth rate was calculated with a N_{final} from day 15 which was determined as the end of the exponential growth phase. Growth rates were also calculated from exponential regression using the data of cell density.

Determination of particulate carbon and nitrogen. Two samples from each replicate were filtered onto precombusted glass fiber filters (Whatman GF/F) and dried at 60°C to measure POC, PIC and PON. One of the two filters was measured on a Carlo Erba Strumentazione Nitrogen Analyzer 1500 to determine the mass of total particulate carbon (TPC). The other filter was fumed for 4h with 37% HCl in a desiccator (Lorrain et al. 2003) and dried at 60°C to measure POC and PON. PIC was calculated as the difference between TPC and POC.

Production rate of POC, PIC, and PON was calculated by multiplying the respective cell content with the cell-specific growth rate.

Coccolith carbon content and morphology. Between 10 - 40 ml were filtered for each sample onto a cellulose acetate filter with a pore size of 0.8 μm (Sartorius) and dried at 60°C for 16 hours for subsequent SEM image analysis. Thereafter pieces of the filters were sputter-coated with gold-palladium and imaged with a Philips XL-30 digital scanning field-emission electron microscope. Attached and detached coccoliths were counted from SEM images. Because whole coccolithophores were on the filters, only the coccoliths on the visible (upper) side of the cells could be counted and the number of coccoliths on the reverse side of the cells had to be estimated by the size of the cell and the placement of the visible coccoliths. 500 - 1200 coccoliths (both attached and detached) per replicate were counted. Coccoliths per liter were calculated from counts, filter area and filtered volume.

From the coccoliths per liter and PIC concentration the average carbon content per coccolith was calculated.

Coccoliths on each filter were also classified in six categories based on their morphology: normal, incomplete, malformed, incomplete and malformed, deformed, and filled. Reference images for each class are presented in Fig. 15. Approximately 100 coccoliths per replicate were analyzed and the percentage of coccoliths in each class determined.

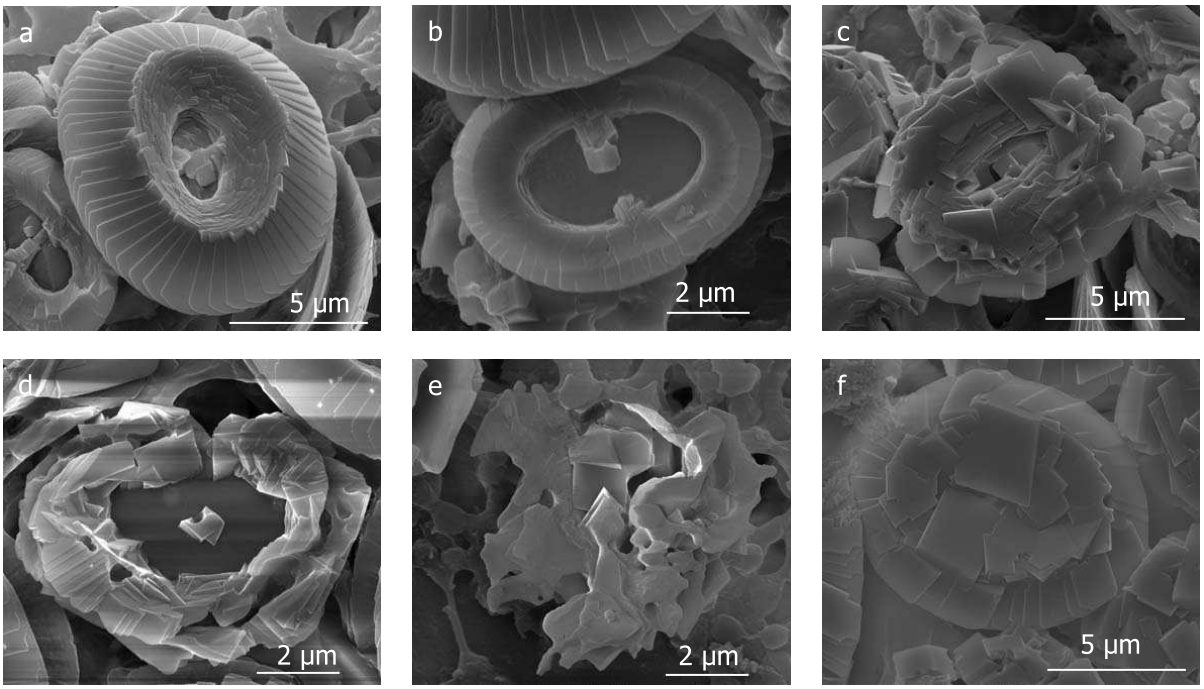

Figure 15: Morphological classification of coccoliths. Images of the six classes of coccoliths. Normal (a), incomplete (b), malformed (c), incomplete and malformed (d), deformed (e), and filled (f) coccoliths.

RESULTS

Media. The carbonate chemistry of the media changed during the growth of the algae. TA, DIC, pCO_2, and the saturation for calcite (Ω) decreased (Tab. 3), but in a range which ensures no inorganic dissolution of calcite in the cultures.

At the beginning of the experiment the alkalinity was 2436 μmol L^{-1} and dissolved inorganic carbon (DIC) 2146 μmol C L^{-1} in the media (Tab. 3). This produces a pH of 8.26, a pCO_2 of 330 ppm, and a Omega of 4.8. Natural values of North Sea water range during the year between 2040-2195 μmol kg^{-1} for DIC and 206-450 μatm for pCO_2 (Bozec et al. 2006) and shows no differences to the media. Growth and calcification of the cells change the parameter of the carbonate system in the media. Alkalinity and DIC decrease to different amounts in the two treatments and left the range of natural values. But the Omega never decreases above two. The Omega shows the edge between inorganic calcium carbonate precipitation and dissolution. The dissolution starts below a Omega of one. Because in all treatments the Omega was above two, it ensures no inorganic dissolution of calcium carbonate in the cultures.

Nutrient concentrations (inorganic or organic) at the end of the experiment were above

Table 3: Carbonate system of the media. pH, pCO_2, and the saturation for calcite (Ω) calculated by the program CO2sys (Lewis & Wallace 1998). TA total alkalinity, DIC dissolved inorganic carbon, T_0 beginning of the experiment, N = nitrate and U = urea treatment at the end of the experiment.

treatment	TA (μmol L^{-1})	DIC (μmol L^{-1})	pH	pCO_2 μatm	Ω
T_0	2436	2146	8.26	330	4.77
N	2141	1749	8.47	155	6.06
U	1970	1719	8.26	165	3.83

Table 4: Nutrient concentrations of the media. The concentration of urea is calculated by the difference of the DON concentrations at the end of the experiment and the natural DON concentration of the North Sea water(DON concentration at T_0 in the N treatment). Concentrations in μmol N L^{-1} for nitrogen compounds and μmol P L^{-1} for orthophosphate. NO_x = nitrite+nitrate, NH_4^+ = ammonia, PO_4 = orthophosphate, DON = dissolved organic nitrogen including the natural urea. T_0 = beginning and T_{final} =end of the experiment. N = nitrate and U = urea treatment.

treatment	time	NO_x	NH_4^+	DON	urea	PO_4
N	T_0	99.4	0.6	65.0	0.0	7.10
	T_{final}	72.9	0.4	66.2	0.0	4.55
U	T_0	0.2	0.6	148.3	83.9	6.95
	T_{final}	0.2	0.3	142.0	75.8	5.32

70 μmol N L^{-1} nitrogen and 4.5 μmol P L^{-1} phosphorus in the two treatments (Tab. 4). Urea was taken up only half as much as nitrate (Tab. 4).

Cell growth. Cells in the nitrate treatment grew exponentially and reached 10^4 cells ml^{-1} after 8 days (Fig. 16) with a growth rate of 0.43 ± 0.02 d^{-1}. This growth rate is low compared to those measured under comparable conditions in other studies (Stoll et al. 2002, Langer et al. 2006), but equal to those of Houdan et al. (2006). Cells in the urea treatment reached only a cell concentration of $3{\cdot}10^3$ cells ml^{-1} after 15 days (Fig. 16) and a growth rate of 0.09 ± 0.00 d^{-1} (Fig. 17).

Particulate content and production of organic carbon and nitrogen. The cellular POC content in the nitrate treatment of 239 ± 42 pg C $cell^{-1}$ (Tab. 5) does not differ from the POC content of 308 ± 28 pg C $cell^{-1}$ in the urea treatment (t-test, $p \geq 0.05$). But the

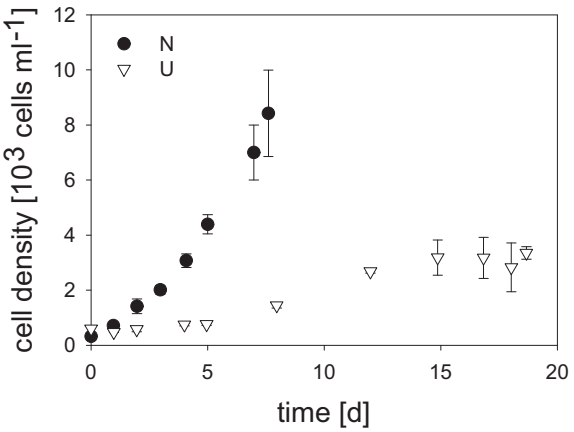

Figure 16: Mean cell abundance vs. time in the two treatments. Error bars show the standard deviation of replicates. N = nitrate and U = urea treatment.

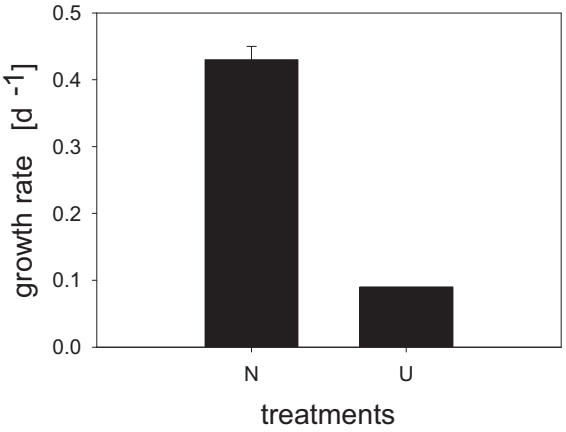

Figure 17: Mean growth rates in the two treatments. Error bars show the standard deviation of replicates. N = nitrate and U = urea treatment.

Table 5: Cellular contents of particulate inorganic carbon, organic carbon , organic nitrogen, and cellular ratios in the two treatments. All ratios in mol:mol. PIC particulate inorganic carbon, POC particulate organic carbon, PON particulate organic nitrogen, N = nitrate and U = urea treatment.

treatment	PIC	POC	PONP
	pg C cell^{-1}	pg C cell^{-1}	pg N cell^{-1}
N	209 ± 28	239 ± 42	42 ± 9
U	815 ± 86	308 ± 28	16 ± 3
	PIC:POC	POC:PON	
N	0.9 ± 0.2	6.7 ± 1.9	
U	2.6 ± 0.4	22.0 ± 4.3	

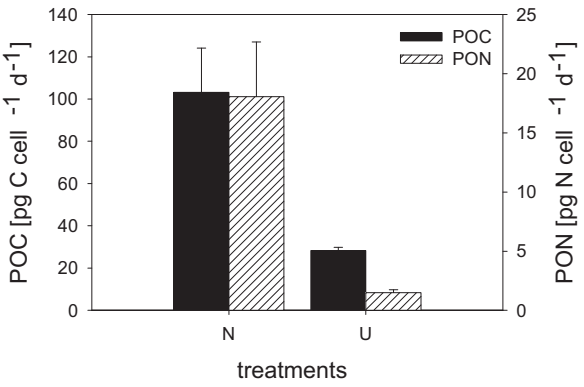

Figure 18: Mean production rates of POC (a) and PON (b) in the two treatments. Error bars show the standard deviation of replicates. N = nitrate and U = urea treatment.

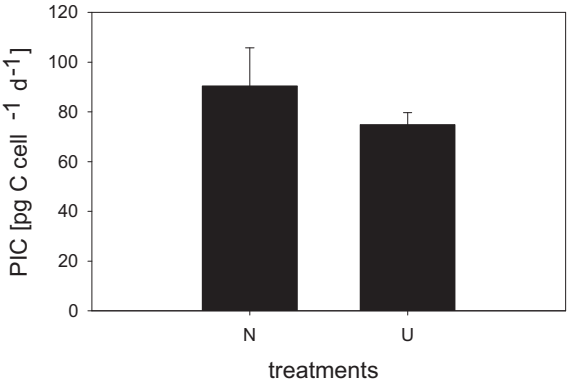

Figure 19: Mean calcification rate (PIC production) in the two treatments. Error bars show the standard deviation of replicates. N = nitrate and U = urea treatment.

PON content per cell shows significant differences between the nitrate (42 ± 9 pg N cell^{-1}) and the urea (16 ± 3 pg N cell^{-1}) treatments (t-test, $p \leq 0.01$) (Tab. 5).

The POC production, however, differed significantly (t-test, $p \leq 0.001$) between the nitrate (103 ± 21 pg C cell^{-1} d^{-1}) and urea (28 ± 2 pg C cell^{-1} d^{-1}) treatment (Fig. 18a). The PON production (Fig. 18b) was 10-fold higher in the treatment with nitrate as in the urea treatment and showed statistical differences (t-test, $p \leq 0.001$).

Consequently differences in the cellular POC:PON ratios were large. The molar POC:PON ratio in the nitrate treatment was 6.7 ± 1.9, whereas cells in the urea treatment showed a statistically different ratio of 22.0 ± 4.3 (t-test, $p \leq 0.001$) (Tab. 5).

Calcification. The PIC content per cell in the urea treatment was significantly higher than in the nitrate treatment (t-test, $p \leq 0.001$). But the calcification rate (PIC production) did not differ significantly (t-test, $p \geq 0.05$) between the treatments (Fig. 19). PIC:POC ratios show differences between the treatments (Tab. 5) due to the different PIC content of the nitrate and urea treatment and the constant POC content of the two treatments (t-test, $p \leq 0.001$). The PIC:POC ratio in the nitrate treatment is 0.9 ± 0.2 and 2.6 ± 0.4 in the urea treatments.

The average inorganic carbon content per coccolith was 21 and 36 pg C coccolith^{-1} in the nitrate and urea treatment, respectively (Fig. 20) and differed significantly (t-test, $p \leq 0.05$). The cells are covered with 10 and 23 coccoliths cell^{-1} in the nitrate and urea treatment, respectively (Fig. 20) and show also significant differences (t-test, $p \leq 0.01$).

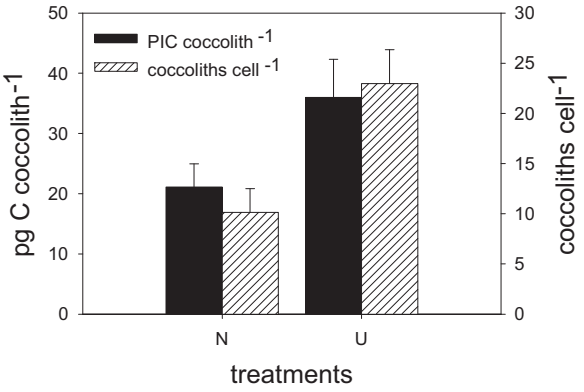

Figure 20: Mean carbon content per coccolith and mean coccoliths per cell in the two treatments. Error bars show the standard deviation of replicates. N = nitrate and U = urea treatment.

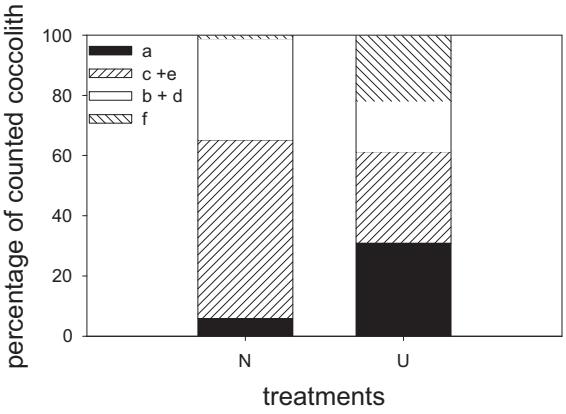

Figure 21: Percentage of normal (a), malformed and deformed (c+e), incomplete and incomplete and malformed (b+d), and filled (f) coccoliths in the two treatments. N = nitrate and U = urea treatment.

Coccolith morphology also varied between treatments (Fig. 21). In the nitrate treatment is a smaller fraction of normal and also of filled coccoliths than in the urea treatment. But there are less incomplete and malformed and incomplete coccoliths in the urea than in the nitrate treatment and also less malformed and deformed coccoliths. In the urea treatment are two times less incomplete and incomplete and malformed coccoliths and also two times less malformed and deformed coccoliths than in the nitrate treatment, but the number of normal and filled coccoliths is six-fold and 19-fold higher in the urea treatment respectively.

DISCUSSION

Nitrogen limitation. C. pelagicus growing on urea is nitrogen limited as inferred from the following observations: compared with the nitrate treatment (1) growth rate is decreased (Fig. 17), (2) PON production is decreased (Fig. 18), and (3) the POC:PON ratio is increased (Tab. 5). But the utilization of urea is co-limit with nickel (Price & Morel 1991) which was not added in this experiment. This co-limitation in our experiment avoid a conclusion about the utilization of urea in *C. pelagicus*. But a nitrogen limitation of the cells is certain of the aforesaid reasons.

Calcification rate under nitrogen limitation. In contrast to the productions of POC and PON, the production of PIC, *i.e.* calcification rate, did not differ between the nitrate

and urea treatment (Fig. 19). What is the advantage of an unabated calcification rate under nitrogen limitation? Reduced growth rates and POC, PON production might lead to an excess of energy in the photosynthetic electron transport chain. Since calcification is an energy consuming process the unaffected calcification rate could provide a means to dissipate excess light energy under nitrogen limitation (Paasche 2002), therewith avoiding photodamage. However, this mechanism is unlikely, because it was recently shown that calcification is not involved in avoiding photodamage under high irradiances in *E. huxleyi* (Trimborn et al. 2007). The actual mechanism by which coccolithophores avoid photodamage is probably not at all connected to calcification, but rather to the xanthophyll or violaxanthin cycle as observed in a diatom (Lohr & Wilhelm 1999).

It might be hypothezised that unabated calcification rate of *C. pelagicus* under nitrogen limitation is not advantageous in a strictly physiological sense. Whether it might be advantageous in an ecological sense is discussed below.

PIC per cell under nitrogen limitation. Calcification rate of *E. huxleyi*, as calculated from growth rate and PIC content per cell, decreases under nitrogen limitation, but the PIC content per cell increases (Paasche 1998). The latter observation is in accordance with our results for *C. pelagicus*. However, the increase in PIC content per cell reported by Paasche (1998) is solely due to an increase in the number of coccoliths per cell. The PIC content of one coccolith even decreased under nitrogen limitation in this study. This is not the case for *C. pelagicus*. In this species, the number of coccoliths per cell as well as the PIC content per coccolith are increased under nitrogen limitation (Fig. 20). Values for PIC per cell and PIC per coccolith fall well within the range of previously reported values (Young & Ziveri 2000, Stoll et al. 2002, Langer et al. 2006). The morphology of the coccoliths was affected as well. The, relative to the normal coccoliths, light coccoliths (termed "incomplete" and "incomplete and malformed") were twice as abundant in the nitrate treatment compared to the urea treatment (Fig. 21). The number of relatively heavy coccoliths (termed "filled") was nearly 20-fold higher in the urea treatment in comparison to cells growing on nitrate (Fig. 21). These morphological data fit the observation that the PIC content per coccolith is higher in the urea treatment (Fig. 20).

In general, coccolith morphology as well as PIC content per cell had been shown to be influenced by carbonate chemistry of seawater (Langer et al. 2006), which was not constant in our experiments (Tab. 3). In *C. pelagicus*, however, coccolith morphology and

calcification rate were insensitive to changes in carbonate chemistry over a wide range of CO_2 concentrations (150 - 900 μatm) (Langer et al. 2006). Therefore the small variability in the carbonate chemistry (Tab. 3) could not have led to a measurable effect on PIC content or morphology.

Ecological and biogeochemical implications of increased PIC per cell. The elevated PIC:POC ratio under nitrogen limitation (Tab. 5) leads to an increase in the overall density of the cell, because calcite is more dense than the protoplast. The higher density of the cell could in turn lead to an accelerated sinking; given that the effect of the higher PIC:POC ratio is not alleviated by floating vacuoles for instance (Young 1994). Elevated sinking velocity under nitrogen limitation was regarded as a means to reach deeper, nitrogen-enriched waters (Baumann et al. 1978, Paasche 2002 and references therein). However, this hypothesis cannot be tested experimentally and therefore can only be assessed on the basis of thorough field research, if at all. From the biogeochemical point of view, accelerated sinking might, at first sight, also be of interest, because it apparently leads to higher export rates of biogenic material. However, the transport of coccolithophore calcite from surface waters to the sea floor is almost entirely mediated by the sinking of fecal pellets (e.g. Honjo 1976). Therefore an increase in the PIC:POC ratio has probably no effect on the export rate of biogenic material, but obviously on the composition of the exported material. The PIC:POC ratio of sinking matter, the so called rain ratio, would increase if *C. pelagicus* was nitrogen limited. Since *C. pelagicus* is a major producer of biogenic calcium carbonate in the northern North Atlantic (Ziveri et al. 2004), this effect might be of importance in this region.

Study IV

Species-specific utilization of organic nutrients by coccolithophores

INTRODUCTION

Phytoplankton growth (e.g. Dugdale 1967, Eppley et al. 1969) as well as biogeographic distribution patterns of phytoplankton (Brand 1994, Winter et al. 1994) depend among other factors on the availability of nutrients. Traditionally nitrate and phosphate are considered as the major nitrogen and phosphorus sources of phytoplankton, although a large pool of organic nitrogen and phosphorus compounds exist in the ocean. Dissolved organic nitrogen (DON) constitutes 89% of the total dissolved nitrogen (TDN) in the surface water of the open ocean (Berman & Bronk 2003). Two of the largest fractions of the DON are urea and the free and combined amino acids (Antia et al. 1991 and references therein). Concentrations of urea in the ocean range between 0.5 μmol L^{-1} in the open ocean and 8.9 μmol L^{-1} in estuary systems (Antia et al. 1991 and references therein). The amino acids, which reach concentrations up to 0.5 μmol per liter in the open ocean (Antia et al. 1991 and references therein) may be divided into non-polar, polar (amino acids with both acidic and basic side chains), acidic, and basic amino acids. The non-polar amino acids seem to constitute the biggest fraction of the amino acids in the ocean, followed by the polar, the acidic and the basic amino acids (Dittmar et al. 2001, Yamashita & Tanoue 2003). Dissolved organic phosphorus (DOP) constitutes up to 75% of the total dissolved phosphorus (TDP) in the ocean and consists mostly of monophosphate esters (55-77%) and nucleotides and nucleic acids (23-45%) (Benitez-Nelson 2000 and references therein). Coccolithophores are unicellular marine algae. They fix calcium carbonate in form of calcite plates (the so-called coccoliths) which cover their cells. Coccolithophores are an important group of phytoplankton in terms of the global carbon and calcium cycles and are believed to be able to utilize organic nutrients (Ietswaart et al. 1994, Palenik & Henson

1997, Waser et al. 1998, Dyhrman & Palenik 2003, Shaked et al. 2006). *Emiliania huxleyi* is in general the best studied species of the coccolithophores and was often used as a key species representing coccolithophores in ecosystem and carbon cycle models (Westbroek et al. 1993, Iglesias-Rodríguez et al. 2002, Moore et al. 2002). However, *E. huxleyi* is a phylogenetic out-group of the coccolithophores (Sáez et al. 2003) and not representative for this group. In terms of calcite export, *E. huxleyi* is, in most areas, outdone by *Coccolithus pelagicus* and *Calcidiscus leptoporus* (Ziveri et al. 2004, Baumann et al. 2004), which are the major calcite producers in the northern North and South Atlantic respectively. This species are typical coccolithophores with regard to phylogeny (Sáez et al. 2003) in contrast to *E. huxleyi*. Differences in the physiological features, like the ability to utilize organic nutrients, between *E. huxleyi* and the typical coccolithophores *C. pelagicus* and *C. leptoporus* are to investigate getting an idea about representative species. In the present study the ability of *E. huxleyi*, *C. pelagicus* and *C. leptoporus* to utilize different compounds of DON and DOP was investigated.

MATERIALS AND METHODS

In total six experiments were conducted monitoring the growth of each of the coccolithophore species on seven different organic nitrogen sources (N-experiments). Other two experiments were conducted monitoring the growth on four different organic phosphorus sources (P-experiments).

Non-axenic monospecific cultures of the three coccolithophores *C. pelagicus* (strain AC400), *C. leptoporus* (strain AC365, both ALGOBANK www.nhm.ac.uk/hosted_sites/ina/CODENET/caencultures.htm), and *E. huxleyi* (strain PML B92/11) were grown in dilute batch culture to study the ability of these species to utilize different nitrogen and phosphorus sources. All cultures were grown at a light intensity of 270 μmol photons m^{-2} s^{-1} in a 16:8 light:dark cycle and a temperature of 17°C. Because *C. pelagicus* grew malformed in artificial seawater and *C. leptoporus* did not grow at all in artificial seawater, all experiments were done in North Sea water after different pretreatments.

N-experiments. In order to remove nitrate, a 22 liter batch of water was 0.22 μm filtered (Durapore Hydrophilic Cartridge, Millipore), autoclaved (121°C for 40 min), and inoculated with *E. huxleyi*, which was grown for four days. Cells and particles were then removed by 0.2 μm filtration (PALL Life Sciences Capsule AcroPak^{TM} 500). Because

E. huxleyi changed the carbonate system by calcification and photosynthesis, the media was air bubbled for five days to restore the media to pH 8. Then the media was 0.2 μm filtered (Nalgene, bottle top filters) into sterilized glass bottles and spiked with vitamins, trace metals (f/4 concentrations), selenium (2.66 μmol L^{-1}), and macro-nutrients at concentrations of 200 μmol N L^{-1} and 14 μmol P L^{-1}. Nitrate was used as a control for the experiment with varying amino acids (AA-experiment). The non-polar amino acids glycine, L-alanine, L-proline, the polar amino acid L-serine, the acidic amino acid L-glutanic acid, and the basic amino acid L-histidine, respectively were added into the seven different treatments of the AA-experiment. These substances are representatives of the large fraction of amino acids in the natural marine DON (Antia et al. 1991 and references therein). Nitrate was also used as a control in the experiment with urea (U-experiment). The treatment with urea was additionally spiked with 100 nmolar nickel to prevent an nitrogen-nickel co-limitation (Price & Morel 1991). Orthophosphate was added to all treatments of the N-experiment as phosphorus source.

P-experiments. Because *E. huxleyi* is able to produce alkaline phosphatase (Riegman et al. 2000), the P-experiments could not be conducted in the some pretreated North Sea water as used in the N-experiments. The coccolithophore species *E. huxleyi* or *C. pelagicus* were inoculated in bottles of 0.2 μm filtered North Sea water and were grown till the natural phosphate of the North Sea water was removed. The so pretreated North Sea water was then treated like the water of the N-experiments. Orthophosphate was used as a control for the P-experiment, and the four treatments with organic phosphorus were spiked with the phosphomonoester glycerophosphate, the nucleotides adenosine monophosphate (AMP), adenosine triphosphate (ATP) and β-nicotinamide adenine dinucleotide (NAD), which are representatives of the largest parts of the natural marine DOP (Benitez-Nelson 2000 and references therein). The P-experiment with *E. huxleyi* was conducted without the AMP source.

Both experiments were divided in two phases. In the first phase (acclimatization phase) the three species were grown twice for one week each time in their respective media to acclimate to the different nutrient sources. This acclimation phase corresponds to 8 to 28 generations depending on nutrient source and species-specific maximum growth rate. Growth rates were monitored during the second phase (the measuring phase) of each experiment in duplicate flasks. At the beginning of the measuring phase all species

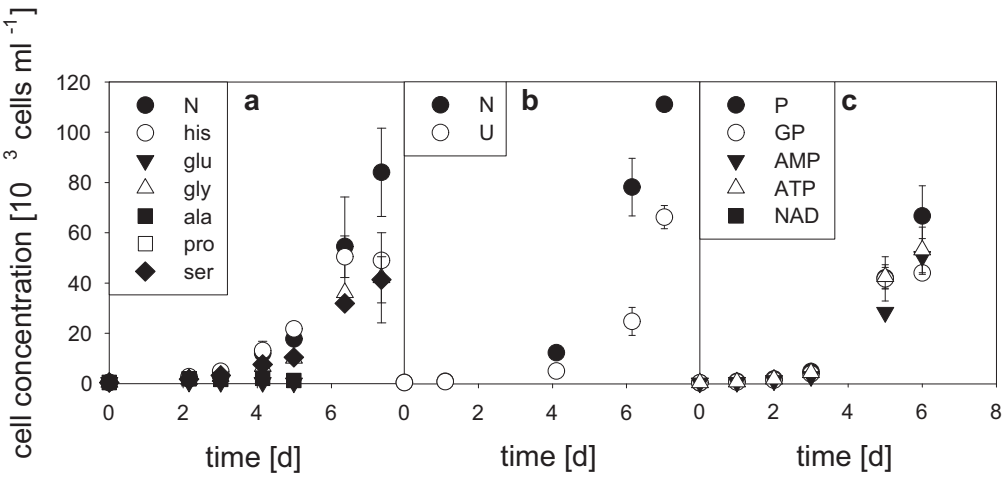

Figure 22: Cell density of *Coccolithus pelagicus* versus time on different nitrogen (a + b) and phosphorus (c) sources. Error bars show the standard deviation of replicates and not visible error bars indicate a smaller error than the symbol. N nitrate, his histidine, glu glutamic acid, gly glycine, ala alanine, pro proline, ser serine, U urea, P orthophosphate, GP glycerophosphate, AMP nucleotides adenosine monophosphate, ATP adenosine triphosphate, NAD β-nicotinamide adenine dinucleotide.

were inoculated in duplicate bottles per treatment with an initial cell concentration of 500 cells ml^{-1}. The measuring phase lasted for 7 - 10 days and cell concentrations were determined every one to three days.

Cells of *C. pelagicus* and *C. leptoporus* were counted with an inverted light microscope (Zeiss, Axiovert 135) using a Sedgewick Rafter Cell from PYSER–SGI. Cells of *E. huxleyi* were counted with a Beckman Coulter Counter. Growth rates μ were calculated from the regression of cell numbers versus time in the exponential growth period. Because the three species exhibited different maximum growth rates, the growth rates on the different organic nutrients were normalized to the growth rates on inorganic nutrients (controls). The growth rate μ is given as an average of the two replicates $\pm$ the standard deviation. Nutrients were measured on 20 ml of a 0.2 μm filtered water sample with an Alliance EVOLUTION^3 Auto-analyzer (Grasshoff et al. 1999)

RESULTS

AA-experiment. During the acclimation phase *C. pelagicus* did not grow on proline and therefore the experiment could not be continued into the measuring phase (no growth

Table 6: Growth rates (μ) of *Coccolithus pelagicus*, *Calcidiscus leptoporus*, and *Emiliania huxleyi* in the different nutrient sources. N = nitrate, gly = glycine, his = histidine, ser = serine, pro = proline, glu = glutamic acid, ala = alanine, U = urea, P = phosphate, GP = glycerophosphate, NAD = β-nicotinamide adenine dinucleotide, AMP = nucleotides adenosine monophosphate, ATP = adenosine triphosphate, n.g. = no growth in acclimation phase, n.m. = treatment not in experiment.

treatment	*Coccolithus pelagicus*	*Calcidiscus leptoporus*	*Emiliania huxleyi*
N	0.79 ± 0.06	0.46 ± 0.00	1.31 ± 0.01
gly	0.72 ± 0.04	0.53 ± 0.00	1.33 ± 0.00
pro	n.g.	n.g.	0.83 ± 0.01
ala	0.07 ± 0.03	n.g.	1.32 ± 0.02
ser	0.62 ± 0.02	n.g.	1.35 ± 0.02
glu	-0.06 ± 0.01	n.g.	0.83 ± 0.01
his	0.70 ± 0.02	0.52 ± 0.03	1.01 ± 0.01
N	0.87 ± 0.02	0.50 ± 0.01	1.17 ± 0.02
U	0.68 ± 0.03	0.44 ± 0.02	0.93 ± 0.00
P	0.89 ± 0.06	n.m.	1.31 ± 0.01
GP	1.01 ± 0.06	n.m.	1.31 ± 0.00
NAD	n.g.	n.m.	1.13 ± 0.00
AMP	0.91 ± 0.01	n.m.	n.m.
ATP	1.00 ± 0.03	n.m.	1.33 ± 0.01

curve in Fig. 22a). Growth of *C. pelagicus* was followed for 7 days (measuring phase) in the remaining amino acid treatments (Fig. 22a), but cells did not grow on glutamic acid and alanine during this phase. Growth rates on the remaining amino acid sources ranged between 0.62 ± 0.02 and 0.79 ± 0.06 d^{-1} (Tab. 6) with the highest growth rate on nitrate.

C. leptoporus did not grow on alanine, proline, serine, and glutamic acid during the acclimation phase (no growth curve in Fig. 23a). This species was observed for 10 days in the measuring phase of the experiment and grew at rates between 0.46 ± 0.00 and 0.53 ± 0.00 d^{-1} (Fig. 23a). Highest growth rate was reached on glycine (0.53 ± 0.00 d^{-1}) (Tab. 6).

E. huxleyi grew in all tested amino acids during both the acclimation and the measuring phase, which lasted 9 days (Fig. 24a). The growth rates ranged between 0.83 ± 0.01 and 1.35 ± 0.02 d^{-1} and *E. huxleyi* grew fastest on serine (1.35 ± 0.02 d^{-1}) (Tab. 6).

U-experiment. *C. pelagicus* had a growth rate of 0.68 ± 0.03 d^{-1} on urea unlike a growth

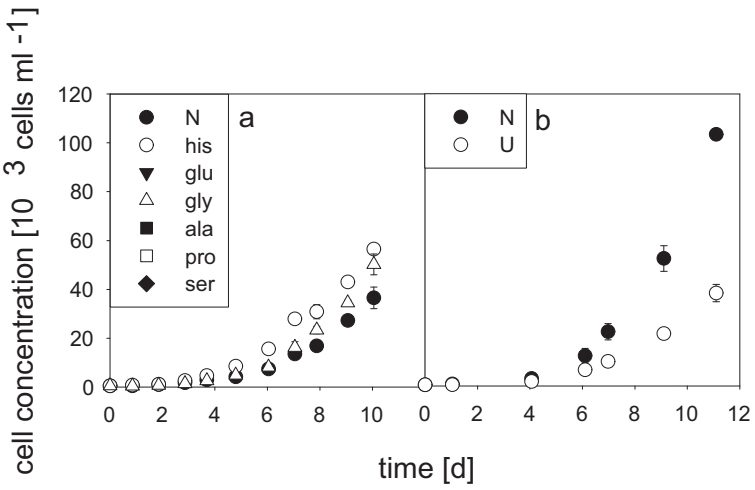

Figure 23: Cell density of *Calcidiscus leptoporus* versus time on different nitrogen sources (a + b). Error bars show the standard deviation of replicates and not visible error bars indicate a smaller error than the symbol. N = nitrate, his = histidine, glu = glutamic acid, gly = glycine, ala = alanine, pro = proline, ser = serine, U = urea.

rate of 0.87 ± 0.02 d^{-1} on nitrate (Fig. 22b). *C. leptoporus* and *E. huxleyi* had also reduced growth rates of 0.44 ± 0.02 and 0.93 ± 0.00 d^{-1} on urea to 0.50 ± 0.01 and 1.17 ± 0.02 d^{-1} on nitrate, respectively (Fig. 23b and 24b).

P-experiment. *C. pelagicus* did not grow on NAD (Fig. 22c), the growth rates on all other tested organic phosphorus sources and orthophosphate were not significantly different between the treatments (ANOVA, $p>0.5$, $n=2$). *E. huxleyi* grew on all tested organic phosphorus sources, but was not tested on AMP (Fig. 24c). Growth rates on the tested phosphorus sources were not significant different expect for the growth rate on NAD (ANOVA, $p<0.001$, $n=2$), this rate was reduced compare to the other rates.

The range of growth rates of the three different species differed due to the different species-specific maximum growth rates. For this reason the growth rates of the three species were normalized to the growth rates on nitrate or phosphate (controls). Because the growth rates in the controls were not always the highest, the normalized growth rates on organic nutrients are sometimes larger than 100%.

The three species grew different on the several nutrient sources (Fig. 25). On the basic amino acid histidine all three species grew well (Fig. 25a), but while *E. huxleyi* and *C. pelagicus* reduced their growth rates, *C. leptoporus* increased its growth rate compare

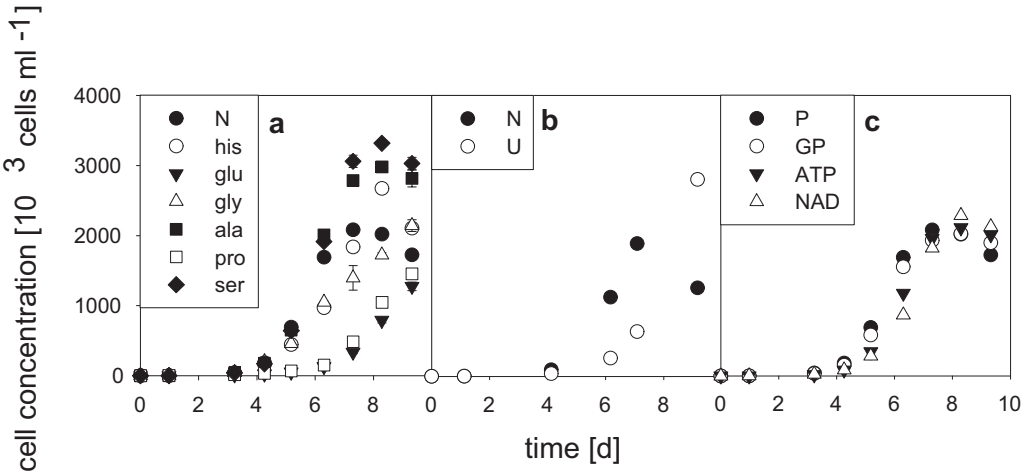

Figure 24: Cell density of *Emiliania huxleyi* versus time on different nitrogen (a + b) and phosphorus (c) sources. Error bars show the standard deviation of replicates and not visible error bars indicate a smaller error than the symbol. N nitrate, U urea, his histidine, glu glutamic acid, gly glycine, ala alanine, pro proline, ser serine, P orthophosphate, GP glycerophosphate, ATP adenosine triphosphate, NAD β-nicotinamide adenine dinucleotide.

to the nitrate treatment. Only the lowest relative growth rate of *E. huxleyi* and the highest of *C. leptoporus* are significant different (ANOVA, $p<0.05$, $n=2$). On the acidic amino acid glutamic acid only *E. huxleyi* was able to grow ($64 \pm 1\%$) (Fig. 25a). The normalized growth on the non-polar amino acids showed large differences (Fig. 25a). All three species grew well on glycine as a nitrogen source with a growth rate of *C. leptoporus* surpassing those on nitrate (Fig. 25a). The highest relative growth rate of *C. leptoporus* differ statistically significant only from the lowest of *C. pelagicus* (ANOVA, $p<0.05$, $n=2$). The other non-polar amino acids alanine and proline supported only the growth of *E. huxleyi*, whereas *C. pelagicus* and *C. leptoporus* grew not at all on alanine or proline (Fig. 25a). On the polar amino acid serine *E. huxleyi* and *C. pelagicus* were able to grow well (with 103 ± 3 and $78 \pm 7\%$, respectively), but with significant different relative growth rates (t-test, $p<0.5$, $n=2$), whereas *C. leptoporus* did not grow on serine.

The growth on urea was reduced by all three species, but growth rates were never less then $78 \pm 4\%$ of those on nitrate (Fig. 25b). The relative growth rates of the three species were thereby not significantly different (ANOVA, $p>0.5$, $n=2$).

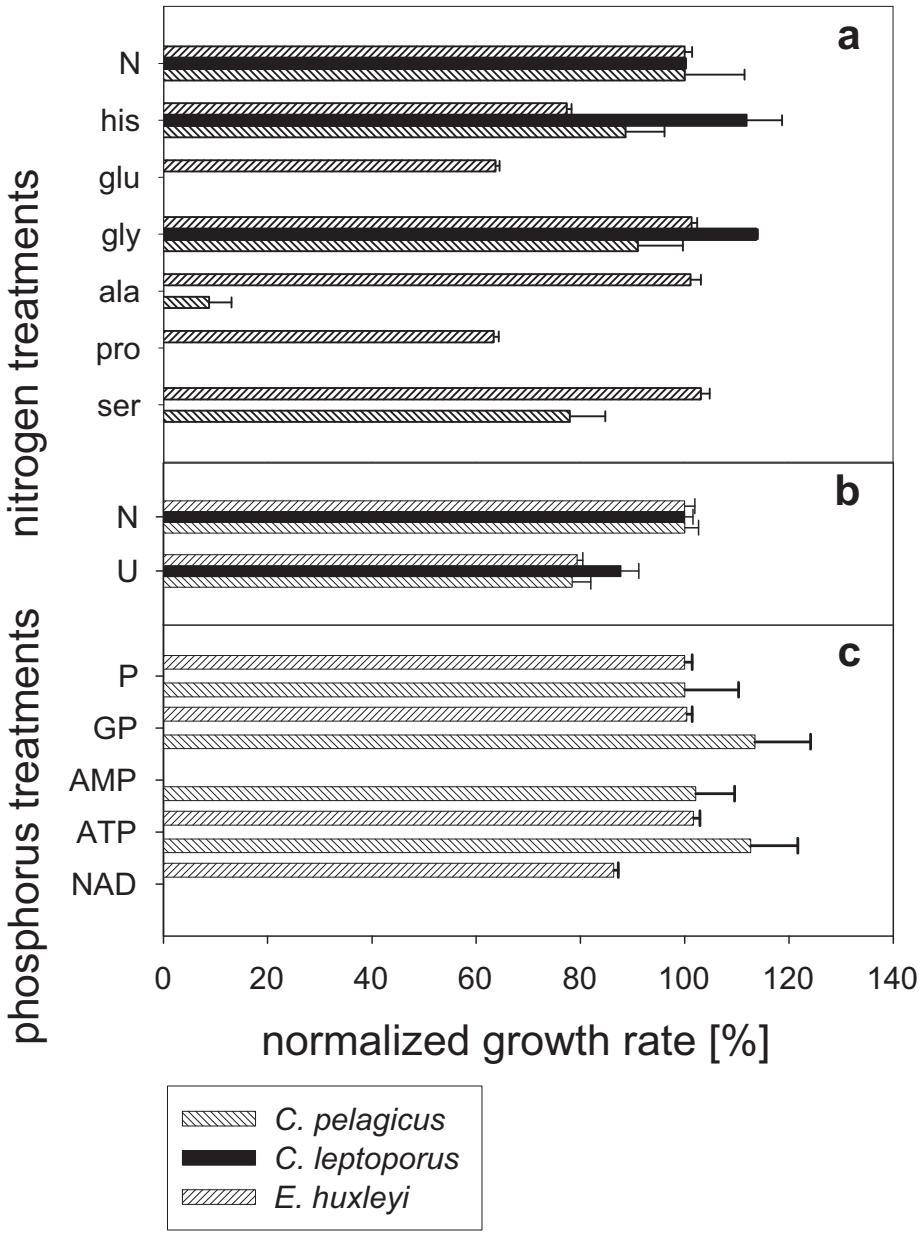

Figure 25: Growth rates of *Coccolithus pelagicus*, *Calcidiscus leptoporus*, and *Emiliania huxleyi* in the different nitrogen (a) and phosphorus (b) sources normalized to the growth rates on inorganic nutrients (controls). Error bars show the standard deviation of replicates and a not visible error bar indicates a smaller error than the symbol. N = nitrate, his = histidine, glu = glutamic acid, gly = glycine, ala = alanine, pro = proline, ser = serine, U = urea, P = orthophosphate, GP = glycerophosphate, AMP = nucleotides adenosine monophosphate, ATP = adenosine triphosphate, NAD = β-nicotinamide adenine dinucleotide.

Both tested species (*E. huxleyi* and *C. pelagicus*) in the P-experiment grew on glycerophosphate and showed no significant differences between the relative growth rates (t-test, $p>0.05$, n=2) (Fig. 25c). The relative growth rate on ATP was also not significant different (t-test, $p>0.05$, n=2). The growth on AMP, another nucleotide, was not tested for *E. huxleyi*, but growth rates of *C. pelagicus* were not significant different in the both sources AMP and ATP (t-test, $p>0.05$, n=2). NAD, however, was not utilizable for *C. pelagicus*; *E. huxleyi* was able to utilize NAD, but with a significant lower growth rate as on the other tested phosphorus sources (ANOVA, $p<0.001$, n=2) (Fig. 25c).

DISCUSSION

The physiology of nutrient uptake. To utilize a nutrient a phytoplankton cell has to take it up and then metabolize it. The nutrient can be transported en bloc and than be metabolized inside the cell using specialized metabolic pathways or alternatively the nutrient is cleaved by ectoenzymes at the cell surface and only the utilizable part is transported into the cell and then metabolized using the regular pathways. Possible utilization pathways of the different tested organic nutrient sources in coccolithophores are discussed in the following section.

Urea, a small, neutral molecule, can diffuse passively into the cell, but urea is also taken up by cells via an active transport system (Antia et al. 1991 and references therein). Once inside the cell urea is cleaved into carbon dioxide and ammonium by an enzyme. It is generally agreed that urease is this enzyme in coccolithophores (Leftley & Syrett 1973). Growth on urea was feasible, albeit at a reduced rate compared to nitrate for different *Emiliania huxleyi* strains (Antia et al. 1975, Palenik & Koke 1995, and our study) as well as for the other two coccolithophores we tested (Fig. 25b). Growth rates on urea are higher than those on nitrate for phytoplankton, which are definitely known to generate the enzyme urease like the diatoms *Thalassiorira pseudonana* and *Thalassiosira weissflogii*, the crysophyte *Aureococcus anophagefferens*, and the dinophyte *Prorocentrum minimum* (Peers et al. 2000, Fan et al. 2003). Reduced growth on urea by coccolithophores compared to these species that generate urease indicate a reduced efficiency of coccolithophores in the utilization of urea. Either the transport of urea into coccolithophore cell is reduced or coccolithophores are not able to metabolize urea equally well. A decreased transport efficiency may be due to the lack of urea transporters in the coccolithophore plasma membrane. A less efficient metabolism may be due to a less production of the enzyme urease

or another way to cleave urea as the other algae producing urease. As it is unknown if coccolithophores have either transport systems or urease, it remains an open question if they lack an efficient transport system or an efficient pathway to metabolize urea. But the reduced growth efficiency on urea suggests, that urea is a less important nitrogen source for the coccolithophores than nitrate.

Amino acids are taken up by three different types of transport systems (Antia et al. 1991), one for basic, one for acidic, and one for non-polar amino acids, respectively. Confusingly the polar amino acids are also taken up by the transport system of the non-polar amino acids, because the polar amino acids have equal numbers of positive and negative charges. Because amino acids are used in every living cell to build proteins, algae are generally able to metabolize amino acids, but not every algae species can utilize every amino acid. Another possibility to utilize an amino acid is the ectoenzymic decomposition by oxidizing the amino acid to hydrogen peroxide, an α-keto acid and ammonium. However, a study by Palenik & Morel (1990) showed no amino acid oxidase activity for *E. huxleyi* strain 12-1. But none of the strains we used was tested for amino acid oxidase. The growth of all three species on the basic amino acid histidine (Fig. 25a) indicates the existence of the transport system for basic amino acids. The different relative growth rates of *C. pelagicus* and *E. huxleyi* may suggest differences in the efficiency while metabolizing histidine. Only *E. huxleyi* grew on the acidic amino acid glutamic acid (Fig. 25a). Because the other common acidic amino acid asparic acid was not tested, it can not be decided if *C. pelagicus* and *C. leptoporus* lack the transport system for acidic amino acids or if they lack the ability to metabolize glutamic acid. The utilization of the non-polar amino acid glycine by all three coccolithophore species (Fig. 25a) indicates that all of them have transport systems for non-polar amino acids (and hence also for the polar amino acids). But the ability to metabolize non-polar or polar amino acids differs between the three coccolithophores. *C. pelagicus* and *C. leptoporus* did not grow on alanine and proline and also not on the polar amino acid serine. However, two different strains of *E. huxleyi* utilized alanine extremely well (Ietswaart et al. 1994 and our study) and showed the highest growth rate on alanine.

There are no known transporters for phosphomonoesters and nucleotides in phytoplankton and thus these compounds of DOP are thought to be utilized via ectoenzymatic degradation. Alkaline phosphatase cleaves phosphomonoesters like glycerophosphate. This

ectoenzyme is very common in marine algae (Kuenzler & Perras 1965) and has also been found in two different strains of *E. huxleyi* (Dyhrman & Palenik 2003, Xu et al. 2006). As both coccolithophore species *C. pelagicus* and *E. huxleyi* grew on glycerophosphate in this study (Fig. 25c) we assume that both produce the ectoenzyme alkaline phosphatase. ATP is thought to be cleaved by the ectoenzyme 5'-nucleotidase which is specialized for the cleavage of nucleotide bounds. *C. pelagicus* and *E. huxleyi* apparently produce ectoenzymes like 5'-nucleotidase to cleave ATP, because both are able to growth on this source (Fig. 25c). Unabated growth on AMP, another phosphomonoester, was also demonstrated by *C. pelagicus* (our study) and *E. huxleyi* (Shaked et al. 2006).

It is more difficult to cleave a phosphate from the molecule NAD, because the phosphorus is embedded in a nucleotide and a nicotine. The transport and/or metabolic way for NAD in phytoplankton used as a nutrient are not known. But regardless if NAD is transported into the cell or enzymatically metabolized outside the cell, NAD has to be cleaved on the nucleotide and nicotine bond to obtain the phosphate. The ability of *E. huxleyi* to grow on NAD (Fig. 25c) indicates that this species has the metabolic pathway to utilize NAD, whereas *C. pelagicus* has it not. Further investigations are needed to discover the metabolism of NAD as a nutrient.

Overall our study indicates that coccolithophores have some general transport systems like those for basic and non-polar amino acids and some general enzymes like alkaline phosphatase. But within the coccolithophores exist also species-specific mechanisms to utilize organic nutrients.

The ecological aspect. Phytoplankton growth rates and thus competitive success of a species is largely determined by environmental factors like temperature, salinity, mixing depth and stratification, light and nutrient availability. The different utilization patterns of organic nutrients by the three coccolithophore species suggest an access to different nutrient sources. *E. huxleyi* was able to grow on all offered nitrogen sources, suggesting that in nature *E. huxleyi* growth is less often limited by nitrogen than the other two coccolithophores. The same is true for phosphorus. Although *C. pelagicus* and *E. huxleyi* utilized phosphomonoester which composes the biggest part of the DOP in the ocean (Benitez-Nelson 2000), they showed differences in the utilization of the other large DOP group, the nucleotides (Fig. 25c). Because *C. pelagicus*is not able to grow on NAD, a part of the dissolved phosphorus in the ocean is perhaps not bioavailable to it.

The disability of *C. leptoporus* to use most of the common organic nitrogen sources (Fig. 25) should increase its dependence on the inorganic nitrogen concentration. This is confirmed by a study of Boeckel et al. (2006), which showed a stronger correlation between the concentration of inorganic nutrients in the surface water and the abundance of *C. leptoporus* coccoliths in sediments than between the nutrients and the abundance of *C. pelagicus* coccoliths in sediments. Studies of Hagino & Okada (2006) and Andruleit & Rogalla (2002) in the equatorial and subequatorial Pacific Ocean and the Arabian Sea showed that *C. leptoporus* does not occur under low inorganic nutrient concentrations, whereas *E. huxleyi* does, supporting the idea that *C. leptoporus* is more dependent on inorganic nutrients than the other two species. All this indicates that *C. leptoporus* occupies a different ecological niche than *E. huxleyi* and *C. pelagicus*. The differences in the utilization of organic nutrients between *E. huxleyi* and *C. pelagicus* also suggest different ecological niches for these two species, but no further studies have been done to substantiate this hypothesis.

The ability of *E. huxleyi* to utilize many organic nutrients provides competitive advantage. Riegman et al. (1992) conducted a competition experiment with *E. huxleyi*, *Phaeocystis* sp. and some diatom species providing inorganic nitrogen and phosphorus at different N:P ratios. *E. huxleyi* outnumbered all other species under low phosphate conditions. The experiment conducted in natural seawater, thus natural organic nutrients must have been present, although they were not measured. A possible explanation for the better growth of *E. huxleyi* could be, that *E. huxleyi* utilized the organic phosphorus compounds of the seawater better than the other algae (Aksnes et al. 1994).

Not only the growth rate and competitive success of a species is determined by environmental factors, its distribution pattern also. Unfortunately little is known about distribution patterns of most coccolithophore species. The published literature shows that *E. huxleyi* is an ubiquitous and cosmopolitan algae (Winter et al. 1994), *C. pelagicus* is most abundant in the North Atlantic (above 45°N), and *C. leptoporus* in the South Atlantic (above 20°S) (Ziveri et al. 2004). Our study shows that nutrient requirements of all three tested coccolithophores differed markedly. Although some studies determine the monomer composition of dissolved amino acids or ATP in different parts of the ocean (Azam & Hodson 1977, Antia et al. 1991, Bronk 2002), currently not enough data exists to investigate the correlation between the distribution patterns and nutrient availability.

However, our study indicates, that we need to monitor organic nutrients in the future to understand distribution patterns and competitive success of different species.

The group of coccolithophores. It is generally agreed that a key species should be biogeochemically relevant and *E. huxleyi* is often used as the key species in matters of calcification. But since a key species is used to represent a whole group it should also be representative for this group. Representing the group of coccolithophore, a species has to fulfill certain criterions. One criterion is the phylogenetic relationship, another the response to environmental conditions. The species response to environmental conditions should reflect the response of coccolithophores in general. However, our study reveals species-specific differences in the ability to utilize organic nutrients. Not only differ *E. huxleyi* in the utilization of organic nutrients from the other two species, but also *C. pelagicus* and *C. leptoporus* differ among each other in our study. The phylogenetic out-group *E. huxleyi* fits to none of the criterions and should not used as a representative species. *C. pelagicus* and *C. leptoporus* have a strong phylogenetic relationship, but show also differences in their response to environmental conditions, not only in the utilization of organic nutrients, but also in their response to changing carbonate chemistry of seawater (Langer et al. 2006). Indicating that the response of one typical coccolithophore reflects not necessarily the response of all typical coccolithophores. Detailed investigations on different geographical origins and phylogenetic positions can reveal if only one coccolithophore species meets all criterions of a representative key species.

Study V

The effect of nickel addition on coccolithophores growing on urea

INTRODUCTION

Urea is a common nitrogen source for many phytoplankton species in the ocean (McCarthy 1972, Antia et al. 1975, Oliveira & Antia 1986, Dyhrman & Palenik 2003). The two enzymes urease and UALase are known to cleave urea inside the cells. Urease occurs in all algal classes except certain orders of green algae (Bekheet & Syrett 1977). The enzyme urease is a nickel metalloenzyme (Dixon et al. 1975) and consequently the growth on urea is co-limited by nickel (Price & Morel 1991). A study of the nickel requirement of twelve marine phytoplankton species during growth on urea indicated a general need of nickel, but in very different amounts (Oliveira & Antia 1986). Hence, Oliveira & Antia suggested that natural nickel concentrations might be too low to support a growth on urea for some species, but appears sufficient for others. Coccolithophores, which were not included in this study, are known to grow on urea (Palenik & Henson 1997, Waser et al. 1998, and study III of this thesis), but nothing is known about a possible nitrogen-nickel co-limitation. Our study fills this gap and determines the growth rates of *Calcidiscus leptoporus*, *Coccolithus pelagicus*, and *Emiliania huxleyi* growing on urea with and without nickel addition.

MATERIAL AND METHODS

Non-axenic monospecific cultures of the three coccolithophores *C. pelagicus* (strain AC400), *C. leptoporus* (strain AC365) (both from ALGOBANK www.nhm.ac.uk/hosted_sites/ina/CODENET/caencultures.htm), and *E. huxleyi* (strain PML B92/11) were grown in batch culture at a light intensity of 270 μmol photons m^{-2} s^{-1} in a 16:8 light:dark cycle and a

temperature of 17°C.

Because *C. pelagicus* grew malformed in artificial seawater and *C. leptoporus* did not grow at all in artificial seawater, natural pretreated North Sea water was used. In order to remove nitrate, a batch of water was 0.22 μm filtered (Durapore Hydrophilic Cartrige, Millipore) and autoclaved (121°C for 40 min). Vitamins and trace metals were added according to f/4 (Guillard 1975) and inoculated with *E. huxleyi* (strain PML B92/11), which was grown for four days. Nitrate concentration was measured on a pre-filtered water sample (syringe filter from Millipore, 0.2 μm pore-size) with a Alliance EVOLUTION^3 Auto-analyzer (Hansen & Koroleff 1999) to assure the complete uptake of nitrate. Cells and particles were then removed from the nitrate-free media by 0.2 μm filtration (PALL Life Sciences Capsule AcroPak^{TM} 500). The carbonate system was restored to its original state by adding sodium bicarbonate (480 μmol L^{-1}) and 0.74 ml of 0.5 molar HCl. Then the media was spiked with vitamins (f/4 concentrations), trace metals (f/4 concentrations), selenium (2.66 μmol L^{-1}), urea (200 μmol N L^{-1}), and orthophosphate (14 μmol P L^{-1}) and used for the experiments.

The experiment comprised two treatments, the urea treatment without nickel addition (U treatment) and the urea plus nickel treatment with a nickel addition of 100 nmol L^{-1} (U+Ni treatment). Our media presumably contained nickel concentrations lower than in situ, as *E. huxleyi* could have utilized some of the nickel.

Before the experiment the three species were grown for 5 to 25 generations, depending on nutrient source and species-specific maximum growth rate in the respective media, to acclimate the culture to the different nutrient sources. During the experiment cell densities of the three species were monitored in duplicate flasks of each treatment.

Cells of *C. leptoporus* and *C. pelagicus* were counted with an inverted light microscope (Zeiss, Axiovert 135) using a Sedgewick Rafter Cell from PYSER–SGI. Cells of *E. huxleyi* were counted with a Beckman Coulter Counter. Growth rates μ were calculated from the regression of cell numbers versus time in the exponential growth period (day 1 to day 11 for *C. leptoporus*, day 1 to day 6 for *C. pelagicus*, and day 1 to day 7 for *E. huxleyi*).

RESULTS

C. leptoporus reached a cell density of 38,000 cells ml^{-1} with and without nickel addition (U and U+Ni treatments) (Fig. 26a) and growth rates of 0.40 $\pm$ 0.00 and 0.44 $\pm$ 0.02 d^{-1},

Table 7: Growth rates of the three species in the different treatments in means ± standard deviation and as percentage of the growth rate on urea plus nickel. Growth rate μ in d^{-1}. U = treatment with 200 μmol N L^{-1} urea and without nickel addition, U+Ni = treatment with 200 μmol N L^{-1} urea and 100 nmol L^{-1} nickel addition.

species	U	U+Ni
Calcidiscus leptoporus	0.40 ± 0.00	0.44 ± 0.02
	91 ± 4%	100%
Coccolithus pelagicus	0.24 ± 0.07	0.72 ± 0.04
	34 ± 9%	100%
Emiliania huxleyi	0.89 ± 0.00	0.93 ± 0.00
	96 ± 0%	100%

respectively (Tab.7). These growth rates were not significantly different (t-test, n = 2, $p > 0.05$).

The growth rate of *C. pelagicus* in the U+Ni treatment was 0.72 ± 0.04 d^{-1} (Tab. 7) and a cell density of 172,000 cells ml^{-1} was reached after 11 days (Fig. 26b). In the U treatment only a cell density of 3,542 cells ml^{-1} and a growth rate of 0.24 ± 0.07 d^{-1} was reached. The growth rates of *C. pelagicus* in both treatments were significantly different (t-test, n = 2, $p < 0.05$).

E. huxleyi (Fig. 26c) reached a growth rate of 0.89 ± 0.00 d^{-1} in the U treatment (Tab. 7), and 0.93 ± 0.00 d^{-1} in the U+Ni treatment. The difference in growth rates between the two treatments was small, but significant (t-test, n = 2, $p < 0.01$).

DISCUSSION

In this experiment pretreated seawater of Helgoland (Germany) was used. Nickel occurs in concentration of 2 - 9 nmol L^{-1} in the western Pacific (Mackey et al. 2002), around 2.4 nmol L^{-1} in the northeast Atlantic (Saager et al. 1997), and nearly 8 nmol L^{-1} around Helgoland (Kremling et al. 1987). The nickel concentration in the treatment without addition was presumably lower than 8 nmol L^{-1}, but in the range of the natural nickel concentrations. A nickel addition of 100 nmol L^{-1} with a urea concentration of 100 μmol L^{-1} was chosen to represent the range of other studies (Oliveira & Antia 1986, Price & Morel

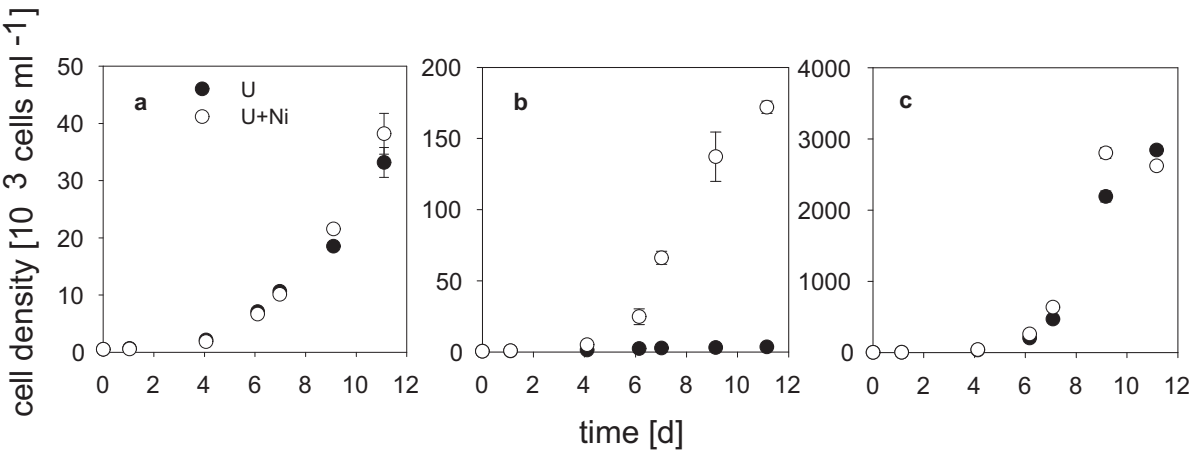

Figure 26: Cell abundance versus time in the different treatments for *Calcidiscus leptoporus* (a), *Coccolithus pelagicus* (b), and *Emiliania huxleyi* (c). Error bars show the standard deviation of duplicates. U = treatment with 200 μmol N L^{-1} urea, U+Ni = treatment with 200 μmol N L^{-1} urea and 100 nmol L^{-1} nickel.

1991, Peers et al. 2000, Dyhrman & Palenik 2003).

C. leptoporus and *E. huxleyi* did not grow differently on urea with and without nickel addition (Tab. 7). Even though the difference in the growth rates with and without nickel addition are statistically significant for *E. huxleyi*, a difference of 0.04 d^{-1} is not enough to reflect real differences in the natural range of growth rates in this species. The measured growth rates indicates that nickel concentrations lower than 8 nmol L^{-1} allow the effective utilization of urea for *C. leptoporus* and *E. huxleyi*. A Ni-N co-limitation under natural conditions is unlikely for these two species.

It is commonly assumed that prymnesiophyceae produce urease (Bekheet & Syrett 1977), although this has not been tested for the species we used. The low requirement of nickel by *C. leptoporus* and *E. huxleyi* could also indicate their use of UALase which does not require nickel for its catalytic activity, rather than urease.

Growth rates of *Co. pelagicus* on urea were reduced without nickel addition compared to growth after nickel addition. *C. pelagicus* growing on urea without nickel addition reached only 34% of the growth rate on urea with nickel addition, indicating a possible Ni-N co-limitation of this species in the ocean.

Similar co-limitations have been found in some diatom species. The growth rate of the

diatom *Phaeodactylum tricornutum* on urea without nickel addition was 27% lower compared to the growth rate on urea after a nickel addition of 2 nmol Ni per μmol urea (Rees & Bekheet 1982). The growth rates of the diatom species *Thalassiosira weissflogii*, *Thalassiosira nordenskioldii*, and *Skeletonema costatum* decreased by 16%, 24%, and 6%, respectively, if no nickel was added compared to those after nickel additions of 5 to 100 nmol per mmol urea (Oliveira & Antia 1986). The presence of nickel apparently influences the growth rates of several species differently. The requirements for nickel that allow an effective growth on urea differ with phytoplankton species (see Rees & Bekheet 1982, Oliveira & Antia 1986, and this study). The occurrence of a Ni-N co-limitation under a certain nickel concentration is therefore extremely species-specific.

3 Discussion

The rising atmospheric pCO_2 and the concomitant changes in global temperatures will change the marine environment, e.g. the carbonate system, sea surface temperature (SST), nutrient and light availability. In this thesis, the effect of the combined increase of seawater CO_2 concentration and SST as well as the impact of different nutrient sources on a phytoplankton community in the North Atlantic was studied. Further, the influence of nutrient availability on growth and calcification of coccolithophores was studied. In the following the results of the different studies are discussed with regard to their role in phytoplankton ecology and possible implications for biogeochemical cycling. Finally, perspectives for future research are given.

3.1 Phytoplankton community composition

Opal generating diatoms, the DMSP producing flagellate *Phaeocystis* which forms mucilagenious colonies, and calcium carbonate producing coccolithophores play different roles in marine ecosystems and elemental cycling. These groups constitute therewith different functional groups according to Falkowski & Raven 1997. Changes in marine primary production, phytoplankton community composition and succession will impact biogeochemical cycles of elements, such as carbon, nitrogen, and silicon (Falkowski et al. 2003). But environmental conditions like nutrient concentrations and ratios and the temporal variability of nutrient concentrations in turn influence, phytoplankton assemblage and primary production (Hutchinson 1961, Gaedeke & Sommer 1986, Tortell et al. 2002).
The response of a natural phytoplankton community of the North Atlantic to combined changes in temperature and CO_2 concentration and to a decreasing inorganic to organic nutrient ratio, which is predicted due to an enhanced stratification in the future, was investigated in studies I and II of this thesis. The North Atlantic is an important region for the oceanic carbon cycle and a large sink for atmospheric CO_2 (Robertson et al. 1993). The above-named functional groups all occur in this region (Weeks et al. 1993, Tyrrell & Taylor 1996, Wassmann et al. 2005 and references therein). Temperature and CO_2 concentration seem to influence the community assemblage and composition (study I of this thesis), whereas an increasing organic to inorganic nutrient ratio does not seem to influence the relative abundance of these functional groups (study II of this thesis). To

what extent the availability of organic nutrients influences the phytoplankton community when nitrate and phosphate are no longer available has to be studied in future experiments.

An elevated CO_2 concentration coupled with sea surface warming seems to favor marine coccolithophores, whereas the sole increase of pCO_2 seems to favor marine diatoms (study I of this thesis). The abundance of the coccolithophore *Emiliania huxleyi* in a coastal Norwegian phytoplankton assemblage was reduced under elevated CO_2 concentrations compared to ambient CO_2 concentrations (Schneider 2004). The results of both studies suggested that only the combination of increasing CO_2 concentration and temperature benefit coccolithophores. Laboratory experiments with *E. huxleyi* under enhanced CO_2 concentration and under enhanced CO_2 concentration and temperature showed similar results (Zondervan et al. 2002, Feng et al. 2008). The growth rate of *E. huxleyi* was independent from the CO_2 concentration, but controlled by light and temperature (Zondervan et al. 2002, Feng et al. 2008). The interpretation of increasing temperature on growth rates on a species level is difficult, because each strain has its optimum temperature and specific temperature range (Brand 1982; 1994). If the used temperature of the experiments equals the optimal temperature a change in temperature will always reduce the growth rate. The transfer of the response of strains on increasing temperature to a natural phytoplankton community is also difficult, because changes in temperature in a defined region may cause shifts of strain dominances depending on their temperature optimum.

The POC production of the North Atlantic phytoplankton community was enhanced under increased CO_2 concentration and temperature, whereas the PIC production of the phytoplankton community was reduced (study I of this thesis), although this treatment contained the highest abundance of coccolithophores. The reduced PIC production may decrease the potential release of CO_2 to the atmosphere in this region as a consequence of calcification (see Zondervan et al. (2001) and equation 1 in the introduction). The reduced PIC production in combination with the enhanced POC production may also decrease the PIC:POC ratio. A reduced PIC:POC ratio may also reduce the potential CO_2 release to the atmosphere in this region (as modelled in Zondervan et al. (2001)), if the quantity of export production is not affected by this altered ratio.

3.2 Nutrient limitation and calcification

Aside from CO_2 and temperature, growth and calcification could also be impacted by nutrient availability. Phosphate and nitrate limitation leads to an increase in the ratio of calcification to photosynthesis in the coccolithophore *Emiliania huxleyi* (Zondervan 2007 and references therein). But which part of the PIC:POC ratio is changed? Although the increase of the calcification to photosynthesis ratio is misleadingly often equated with an increase in the 'calcification' (e.g. Berry et al. 2002, Sciandra et al. 2003), the term 'calcification' is used in very different ways. 'Calcification' is sometimes correctly equated with the calcification rate, but sometimes also incorrectly equated with the inorganic carbon content per cell. Hence, these studies are not comparable. If one examines the calcification rate and the photosynthesis rate (often determined as POC production in literature) of different studies, the calcification rate as well as POC production of *E. huxleyi* decreased under nitrate limitation (Paasche 1998, Riegman et al. 2000). The increase in the calcification to photosynthesis ratio is caused by a stronger decrease in POC production than in calcification rate. However, under phosphorus limitation the calcification rate of *E. huxleyi* did either not change (Paasche 1998, Riegman et al. 2000) or increase (Paasche & Brubak 1994) in the literature, whereas the POC production always decreased. *Coccolithus pelagicus*, one of the heaviest calcifying living coccolithophores and probably one of the most important species in terms of calcite export to the sediment (Broerse et al. 2000, Ziveri et al. 2000), did not change calcification rate in response to nitrogen limitation, whereas the POC production decreased (study V of this thesis). Nutrient limitation seems to influence the calcification rate differently depending on nutrient source and species. Perhaps even strains are differently influenced, Paasche & Brubak (1994) and Riegman et al. (2000) measured different trends in the calcification rate under phosphorus limitation on two different strains of *E. huxleyi*.

Hence, one question is how nutrient limitations influence the mechanisms of calcification and photosynthesis. A decreasing POC production under nutrient limitation is not surprising, because nitrogen is a component of proteins and nucleic acids in cells (Geider & La Roche 2002) and phosphorus is not only an essential component of nucleic acids in cells, but is also involved in the energetic processes via molecules like ATP or NADPH (Dugdale 1967, Geider & La Roche 2002). Phosphorus limitation seems also to affect the CO_2-concentrating mechanism (CCM) and limit photosynthetic CO_2 fixation and

algal growth (Beardall et al. 1998). The above-mentioned results of in- or decreasing calcification rates in response to nutrient limitation suggest that the calcification rate of coccolithophores is less dependent on nitrogen and phosphorus than cell division. How nitrogen or phosphorus concentrations could influence the calcification on a cellular level has not been resolved. Coccolith-formation is a complex process and includes many different steps like the transport of calcium and bicarbonate into the coccolith vesicle or the clearly defined growth of the $CaCO_3$ crystals (Young et al. 1999). The different reactions of phosphorus and nitrogen limitation on the calcification rate of some coccolithophore species (Paasche & Brubak 1994, Paasche 1998, Riegman et al. 2000 and study V of this thesis) indicate that calcification processes and biomass building processes in a coccolithophore cell are not directly linked. Calcification is uncoupled from growth rate in *C. pelagicus* (study V of this thesis). Also Corstjens & González assumed that the synthesis of proton-pumping V-type ATPase in a coccolith vesicle membrane could be continued after the stop of cell division and should therefore be independent from growth rate. The expression of the gene, encoding a subunit of this ATPase, was constitutive under both repleted and limited nitrogen and phosphorus conditions (Corstjens & González 2004). However, the production of coccoliths occurs inside the cell and has to be coupled to cell metabolism somehow. The absolute requirement for nitrogen in calcification (Merrett et al. 1993) illustrated this assumption. The understanding how nitrogen and phosphorus influence the process of calcification inside the cell is a basic requirement for predictions of the PIC:POC ratio under nutrient limitation of coccolithophore species.

3.3 Utilization of organic nutrients

Nitrogen and phosphorus limitation are usually related to a nitrate or orthophosphate limitation, but to what extent the utilization of organic nutrients prevent these deficits is inadequately studied. To assess the response of different phytoplankton taxa to nutrient deficit, it is therefore necessary to know whether organic nutrients can be utilized, and if so, what kinds of organic nutrients.

Study IV of this thesis has shown that many coccolithophores are able to utilize a range of different organic nutrients like urea, amino acids, and phosphomonoesters, but the utilization is species-specific. *Calcidiscus leptoporus* grew only in one nonpolar and the basic amino acids of six tested amino acids, *Coccolithus pelagicus* was not able to grow

on most of the nonpolar and the acidic amino acids, whereas *Emiliania huxleyi* grew in all tested amino acids. *E. huxleyi* grew also in all tested organic phosphorus compounds, whereas *C. pelagicus* was not able to utilize nicotinamide adenine dinucleotide (NAD). Information about the ability of phytoplankton species to utilize different compounds of DON or DOP could help to explain distribution patterns and succession, but future studies on distribution and concentration of the different organic nutrient sources in the ocean are necessary to combine the physiological studies with observed distribution and succession.

A phytoplankton community from the North Atlantic growing on different organic to inorganic nutrient ratios showed no differences in the percentage of the abundance of functional groups (study II of this thesis). Nevertheless, some diatom genera seemed to prefer either inorganic or organic nutrients (study II of this thesis). *Fragilariopsis* spp. had higher growth rates on phosphate compared to glycerophosphate, a phosphomonoester. Phosphomonoesters are thought to be utilized by the ectoenzyme alkaline phosphatase (APase). If *Fragilariopsis* cannot utilize glycerophosphate, this genera could perhaps not be able to produce APase. A decreasing inorganic to organic nutrient ratio due to a stronger stratification may drive these genera into a phosphorus limitation, because of its inability to utilize a large fraction of DOP. Silicon content per cell as well as Si/POC ratio increased in *Thalassiosira pseudonana* under phosphorus limitation in a laboratory experiment (Claquin et al. 2002). If phosphorus limitation also influences the Si content per cell and the Si/POC ratio by *Fragilariopsis* spp., a decrease of the inorganic to organic nutrient ratio may influence the elemental composition of exported material in the ocean. To what extent the growth of other diatom genera or species is influenced by the ratio of inorganic to organic nutrients has to be studied in future experiments.

The assimilation of some organic nutrients is dependent on micro-nutrients. Study V of this thesis analyzes the requirement of nickel during the utilization of urea. The degree of this requirement is species-specific, *C. pelagicus* needed higher concentrations of nickel than *C. leptoporus* and *E. huxleyi* for maximum growth rates. *C. leptoporus* and *E. huxleyi* seem to manage the utilization of urea on natural nickel concentrations ($\sim$0.5 - 9 nmol L^{-1} (e.g. Kremling et al. 1987, Saager et al. 1997, Mackey et al. 2002)), whereas a Ni-N co-limitation of *C. pelagicus* under natural conditions is conceivable even if nickel occurs in high natural concentrations. To assess the influence of this co-limitation on the growth

of *C. pelagicus* in the ocean, ambient nitrogen and nickel concentrations should to be measured during *C. pelagicus* blooms in the future.

The enzyme alkaline phosphatase (this enzyme cleaves phosphate from its organic moiety (Kuenzler & Perras 1965, Cembella et al. 1984)) is a Zn metalloenzyme (McComb et al. 1979, Cembella et al. 1984) and for this reason another example of a potential limitation of a micro-nutrient coupled to the utilization of organic nutrients. Riegman et al. (2000) concluded that such a co-limitation in *E. huxleyi* is only in a few areas possible due to the most efficient phosphatases among eukaryotic oceanic phytoplankter in *E. huxleyi* (Shaked et al. 2006). It has to be studied to which extent a Zn-P co-limitation is possible in other coccolithophore species or other phytoplankton species in general with perhaps less efficient phosphatases.

3.4 Perspectives for future research

Coccolithophores influence the marine carbon cycle by both photosynthesis and calcification. The species *Emiliania huxleyi* is studied extensively, but this species is not only a phylogenetic out-group (Sáez et al. 2003), it is only one of several important coccolithophore species (e.g. *Coccolithus pelagicus*, *Calcidiscus leptoporus*, *Helicosphaera carteri*) in terms of calcite export (Ziveri et al. 2004, Baumann et al. 2004). Hence, it is important to study the effects of environmental conditions on the other species. In the investigations presented here (Langer et al. 2006, study III, IV, and V of this thesis) not all growth factors have been assessed. To understand consequences of the possible decrease of inorganic nutrient concentrations in the future due to a stronger stratification, the effect of nitrogen limitation on growth and calcification in *C. leptoporus*, as well as the effect of phosphorus limitation on growth and calcification in *C. pelagicus* and *C. leptoporus* still need to be studied in future experiments.

Also some experiments were done on the effects of combined growth factors on *E. huxleyi* and/or *Gephyrocapsa oceanica*. Sciandra et al. (2003) showed the combined effect of nitrate limitation and high CO_2 concentration on photosynthesis and calcification rate in *E. huxleyi*. The inhibiting effect of high CO_2 concentration on calcification rate and the reduced POC production under nitrate limitation seemed to balance the PIC to POC production ratio. The response to varying CO_2 concentrations and light regimes of *E. huxleyi* and *Gephyrocapsa oceanica* was also studied by Zondervan et al. (2002), Schneider

(2004). They observed a correlation between the degree of calcification and light intensity. Also the effect of the combination of increasing pCO_2, temperature, and irradiance level on *E. huxleyi* has been studied (Feng et al. 2008). An increase of the irradiance level reduced the PIC content per cell, this effect was boosted under high CO_2 conditions (Feng et al. 2008). Combined effects of changes in temperature, CO_2 concentration, light, and nutrient availability on coccolithophores have to be studied in future experiments. Furthermore, experiments on the effects of combined growth factors are needed to decide which environmental conditions have the strongest impact on growth and calcification in different important coccolithophore species like *E. huxleyi* or *C. pelagicus*.

The utilization of organic nutrients is another growth factor which needs further studies. The general utilization of DON and DOP is known for several phytoplankton species of all groups (Cembella et al. 1984, Palenik & Morel 1990, Antia et al. 1991, Ietswaart et al. 1994, Huang & Hong 1999, Peers et al. 2000, Berg et al. 2003, Lomas 2004, Yamaguchi et al. 2005, Shaked et al. 2006), but less is known about the ability to utilize particular compounds of DON or DOP. To assess the significance of these nutrients in the ocean, we have to investigate their bioavailability in field studies. Concentrations in the ocean, metabolic pathways, and uptake rates are the information we need to assess the significance of DON or DOP compounds in the ocean.

Organic nutrients originate inter alia from phytoplankton release, this release is dependent on physiological stress, temperature, and light level (Bronk 2002). Changing conditions due to the increasing CO_2 concentrations may influence the concentrations and composition of the different organic nutrient sources. Berman & Bronk noticed 2003 that '... little is known about the interplay of various DON sources that are used by phytoplankton ...' and the same statement applies to DOP sources. Studies have shown that ammonia concentrations can influence the uptake rate of nitrate by phytoplankton (Eppley et al. 1969, Lomas & Glibert 1999, Varela & Harrison 1999), to what extent the different DON and DOP compounds can influence their uptake among each other has not been tested yet.

This thesis presents a further step in the understanding of the utilizability of organic nutrients. However, many questions are still open and more investigations are necessary to understand the role of the organic nutrients in the ocean completely.

4 Summary

This thesis investigated the ability of marine phytoplankton (especially coccolithophores) to utilize organic nutrients and the influence of CO_2-induced changes of environmental conditions like a decreased ratio of inorganic to organic nutrients on a phytoplankton community.

The effect of the combined increase of CO_2 concentration and temperature on a North Atlantic phytoplankton community was studied in a chemostat experiment (study I). The initial phytoplankton assemblage of diatoms, coccolithophores, and chrysophyta was at the end of the experiment dominated by chrysophyta under enhanced temperature, by diatoms under enhanced CO_2 concentration, and by coccolithophores under enhanced temperature and CO_2 concentration. The coccolithophore dominated aassemblage showed an increased particulate organic carbon (POC) production, whereas the particulate inorganic carbon (PIC) production was reduced compare to the other assemblages. The combined increase of CO_2 concentration and temperature may therefore reduce the potential CO_2 release to the atmosphere in the North Atlantic as a consequence of reduced calcification and decreased PIC:POC ratio.

Another study on a North Atlantic phytoplankton assemblage had no effect on the composition of the initial phytoplankton assemblage consisting of *Phaeocystis* spp., diatoms, and dinoflagellates under a decreased ratio of inorganic to organic nutrients (study II), but showed some genera specific preferences for either inorganic or organic nutrients. The ability of genera to utilize organic nutrients (like *Chaetoceros* spp. and *Rhizosolenia* spp. in this experiment) indicates a competitive advantage of these genera compared to inorganic nutrient preferring genera like *Fragilariopsis* spp. when organic nutrients are available.

The coccolithophore *Coccolithus pelagicus* was nitrogen limited when grown on urea (study III) which cannot be utilized without nickel addition. PIC content per cell was higher in the urea treatment compared to the nitrate treatment and the coccolith morphology shifted to a higher fraction of presumably heavy coccoliths in the urea treatment. However, the PIC production was constant. A higher PIC content per cell may cause a higher PIC:POC ratio of sinking matter in regions where *C. pelagicus* is a major biogenic calcium carbonate producer like known for the northern North Atlantic.

Three coccolithophores (*Emiliania huxleyi*, *C. pelagicus*, and *Calcidiscus leptoporus*) were

grown on seven different organic nitrogen compounds and three different organic phosphorus compounds (study IV) to investigate if the utilization of organic nutrient compounds is species-specific. All three coccolithophores were able to utilize a range of different organic nutrients, but *C. pelagicus* did not grow on NAD, a fraction of dissolved phosphorus in the ocean, and *C. leptoporus* did not grow on most of the amino acids which are common organic nitrogen sources. This disability could indicate their dependence on nitrate and phosphate and lead to a competitive advantage over other species which are able to utilize these organic nutrients.

The possibility of a nitrogen-nickel co-limitation of the coccolithophores *E. huxleyi*, *C. pelagicus*, and *C. leptoporus* growing on urea in the ocean was investigated in study V. All three species were grown on urea with or without nickel addition. *E. huxleyi* and *C. leptoporus* grew with equal growth rates in both treatments, but the growth rate of *C. pelagicus* in the treatment without nickel addition was reduced by 66 ± 9% compared to the growth rate in the treatment with nickel addition. *E. huxleyi* and *C. leptoporus* seem to be able to utilize urea under natural nickel concentrations, whereas a Ni-N co-limitation of *C. pelagicus* under natural conditions is conceivable even if nickel occurs in high natural concentrations.

The results of this thesis are a further step to understand the impact of CO_2-induced changes of environmental conditions on the marine phytoplankton. Changes in CO_2 concentration and temperature modify phytoplankton community composition. The species-specific utilization of organic nutrient compounds as well as N-Ni co-limitation of coccolithophores also suggest an impact of organic nutrients on the composition of a phytoplankton community. Furthermore, species which are not able to utilize organic nutrients may be nitrogen or phosphorus limited under a decreased inorganic to organic nutrient ratio. These limitations may affect silicification and calcification and therefore change biogeochemical cycles.

5 Zusammenfassung

Diese Arbeit befasst sich mit der Nutzbarkeit organischer Nährstoffe für marine Phytoplankter, insbesondere für Coccolithophoriden. Des Weiteren werden in ihr die Auswirkungen der sich verändernden Umweltbedingungen auf eine Phytoplanktongemeinschaft untersucht.

Die Auswirkungen der kombinierten Erhöhung von CO_2-Konzentration und Temperatur wurden an einer Phytoplanktongemeinschaft des Nordatlantiks in einem Chemostat-Experiment untersucht (Untersuchung I). Von einer anfänglichen Gemeinschaft aus Diatomeen, Coccolithophoriden und Chrysophyten setzten sich die Chrysophyten bei einer erhöhten Temperatur, die Diatomeen bei einer erhöhten CO_2 Konzentration und die Coccolithophoriden bei einer Kombination beider Parameter durch. In der Coccolithophoriden dominierten Gemeinschaft war die POC Produktion erhöht, jedoch die PIC Produktion erniedrigt im Vergleich zu den anderen Gemeinschaften. Die Kombination eines CO_2- und Temperaturanstieges kann daher durch die reduzierte Kalzifizierungsrate und das folglich verkleinerte PIC:POC Verhältnis die potentielle CO_2 Abgabe des Ozeans an die Atmosph"are im Nordatlantik reduzieren.

Eine andere nordatlantische Phytoplanktongemeinschaft, hauptsächlich bestehend aus den Phytoplanktongruppen *Phaeocystis* spp., Diatomeen und Dinoflagellaten, zeigt keine Veränderung der Phytoplanktongruppen zueinander durch ein verringertes Verhältnis der anorganischen zu organischen Nährstoffe (Untersuchung II). Jedoch zeigten einige Gattungen Präferenzen für anorganische oder organische Nährstoffe. Wenn organische Nährstoffe zur Verfügung stehen, sollten Gattungen, die organische Nährstoffe nutzen können (wie *Chaetoceros* spp. und *Rhizosolenia* spp. in dieser Untersuchung), einen Vorteil gegenüber jenen Arten haben, die nur anorganische Nährstoffe nutzen können (wie *Fragilariopsis* spp.).

Das Wachstum der Coccolithophoride *Coccolithus pelagicus* zeigte eine Stickstofflimitierung unter Urea (Untersuchung III). Da jedoch das Wachstum mit Urea durch das Enzym Urease an Nickelkonzentrationen gekoppelt ist (das in dieser Untersuchung nicht zugesetzt wurde), kann nicht bestimmt werden, ob *C. pelagicus* nicht mit Urea wachsen kann oder ob den Zellen Nickel zum Wachstum fehlte. Der PIC Gehalt pro Zelle war mit Urea höher als mit Nitrat und auch die Morphologie der Coccolithen verschob sich in dem Ansatz mit Urea zu schwereren Coccolithen (filled coccoliths). Die PIC Produktion war

jedoch in beiden Ansätzen gleich. Ein höherer PIC Gehalt pro Zelle könnte ein höheres PIC:POC Verhältnis des absinkenden Materiales unter Stickstofflimitierung in jenen Regionen bewirken, in denen *C. pelagicus* ein Hauptproduzent des Kalziumkarbonates ist.
Drei Coccolithophoriden (*Emiliania huxleyi*, *C. pelagicus* und *Calcidiscus leptoporus*) wurden auf eine artspezifische Nutzung organischer Nährstoffe untersucht, indem ihr Wachstum mit sieben verschiedenen organischen Stickstoffquellen und drei organischen Phosphorquellen untersucht wurde (Untersuchung IV). Alle drei Arten konnten verschiedene Quellen nutzen, jedoch konnte *C. pelagicus* kein NAD und *C. leptoporus* viele Aminosäuren nicht nutzen. Dadurch sollte ihre Abhängigkeit von anorganischen Nährstoffen gröβer sein als bei *E. huxleyi*, was ihre Konkurrenzfähigkeit zu anderen Arten, die anorganische Nährstoffe nutzen können, verringert.
Die Annahme einer möglichen Stickstoff-Nickel Co-Limitierung bei Phytoplanktern wurde an den drei Coccolithophoriden *E. huxleyi*, *C. pelagicus* und *C. leptoporus* untersucht (Untersuchung V). Dafür wurde das Wachstum dieser drei Arten an Urea mit oder ohne Nickelzugabe beobachtet. *E. huxleyi* und *C. leptoporus* wuchsen unabhängig von der Nickelzugabe in beiden Ansätzen gleich gut während das Wachstum von *C. pelagicus* ohne Nickelzugabe um $66 \pm 9\%$ reduziert war. Man kann daher annehmen, dass *E. huxleyi* und *C. leptoporus* die natürlichen Nickelkonzentrationen im Ozean ausreichen, um mit Urea zu wachsen, wohingegen *C. pelagicus* nur mit Urea wächst, wenn hohe Nickelkonzentrationen vorhanden sind.
Die Ergebnisse dieser Doktorarbeit sind ein weiterer Schritt zum Verständnis der Auswirkungen des anthropogen bedingten CO_2 Anstieges (und der einhergehenden Veränderungen der Umweltbedingungen) auf das marine Phytoplankton. Veränderungen der CO_2 Konzentrationen und der Temperatur scheinen Phytoplanktongemeinschaften zu verändern. Die hier festgestellte artspezifische Nutzung organischer Nährstoffe und die wahrscheinliche Co-Limitierung des Coccolithophoridenwachstums deutet ebenfalls eine Veränderung der Phytoplanktongemeinschaft unter einem verringerten Verhältnis der anorganischen zu organischen Nährstoffe an. Arten, die keine organischen Nährstoffe nutzen können, wären unter solchen Bedingungen Stickstoff- oder Phosphorlimitiert und könnten eine Veränderung ihrer Silifizierung oder Kalzifizierung zeigen, die wiederum den biogeochemischen Kreislauf verschiedener Element beeinflussen würde.

6 References

Admiraal W, Veldhuis MJW (1987) Determination of nucleosides and nucleotides in seawater by HPLC; application to phosphatase activity in cultures of the alga *Phaeocystis pouchetii*. Mar Ecol Prog Ser 36:277–285

Aksnes DL, Egge JK, Rosland R, Heimdal BR (1994) On the representation of *Emiliania huxleyi* in phytoplankton simulation models. A first appoach. Sarsia 79:291–300

Andruleit H, Rogalla U (2002) Coccolithophores in surface sediments of the Arabian Sea in relation to environmental gradients in surface waters. Mar Geol 186:505–526

Antia NJ, Berland BR, Bonin DJ, Maestrini SY (1975) Comparative evaluation of certain organic and inorganic sources of nitrogen for phototrophic growth of marine microalgae. J Mar Biol Assoc UK 55:519–539

Antia NJ, Harrison PJ, Oliveira L (1991) The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia 30:1–89

Archer D, Winguth A, Lea D, Mahowald N (2000) What caused the glacial/interglacial atmospheric pCO_2 cycles? Rev Geophys 38:159–189

Archer SD, Stelfox-Widdicombe CE, Malin G, Burkill PH (2003) Is sulphide production related to microzooplankton herbivory in the southern North Sea? J Plank Res 25:235–242

Armstrong RA, Lee C, Hedges JI, Honjo S, Wakeham SG (2002) A new, mechanistic model for organic carbon flxes in the ocean based on the quantitative association of POC with ballast minerals. Deep-Sea Res Part II 49:219–236

Azam F, Hodson RE (1977) Dissolved ATP in the sea and its utilisation by marine bacteria. Nature 267:696–698

Babin M, Morel A, Gagnon R (1994) An incubator designed for extensive and sensitive measurements of phytoplankton photosynthetic parameters. Limnol Oceanogr 39:496–510

Balch WM, Drapeau DT, Fritz JJ, Bowler BC, Nolan J (2001) Optical backscattering in the Arabian Sea–continuous underway measurements of particulate inorganic and organic carbon. Deep-Sea Res Part I 48:2423–2452

Baumann FG, Isenberg HD, Jr JG (1978) The Inverse Relatioship Between Nutrient Nitrogen Concentration and Coccolith Calcification in Cultures of the Coccolithophorid *Hymenomonas* sp. J Protozool 25:253–256

Baumann KH, B"ockel B, Frenz M (2004) Coccolith contribution to South Atlantic carbonate sedimentation. In: Thierstein HR, Young JR (eds.) Coccolithophores - From Molecular Processes to Global Impact, Springer, Berlin, pp. 367–402

Beardall J, Johnston A, Raven J (1998) Environmental regulation of CO_2-concentrating mechanisms in microalgae. Can J Bot 76:1010–1017

Bekheet IA, Syrett PJ (1977) Urea-Degrading Enzymes in Algae. Br phycol J 12:137–143

Benitez-Nelson C (2000) The biogeochemical cycling of phosphorus in marine systems. Earth Sci Rev 51:109–135

Berg GM, Glibert PM, Lomas MW, Burford MA (1997) Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. Mar Biol 129:377–387

Berg GM, Jørgensen NOG (2006) Purine and pyrimidine metabolism by estuarine bacteria. Aquat Microb Ecol 42:215–226

Berg GM, Repeta DJ, LaRoche J (2003) The role of the picoeukaryote *Aureococcus anophagefferens* in cycling of marine high-molecular weight dissolved organic nitrogen. Limnol Oceanogr 48:1825–1830

Berman T, Bronk DA (2003) Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. Aquat Microb Ecol 31:279–305

Berman T, Chava S (1999) Algal growth on organic compounds as nitrogen sources. J Plankton Res 21:1423–1437

Berry L, Taylor AR, Lucken U, Rayn KP, Brownlee C (2002) Calcification and inorganic carbon acquisition in coccolithophores. Func Plant Biol 29:289–299

Boeckel B, Baumann KH, Henrich R, Kinkel H (2006) Coccolith distribution patters in South Atlantic and Southern Ocean surface sediments in relation to environmetal gradients. Deep-Sea Res Part I 53:1073–1099

Bopp L, Monfray P, Aumont O, Dufresne JL, Treut HL, Madec G, Terray L, Orr JC (2001) Potential impact of climate change on marine export production. Global Biogeochem Cy 15:81–99

Boyd PW, Doney SC (2002) Modeling regional responses by marine pelagic ecosystems to global climate change. Geophys Res Lett 29:1806, doi: 10.1029/2001GL014130

Bozec Y, Thomas H, Schiettecatte LS, Borges AV, Elkalay K, de Baar HJ (2006) Assessment of the processes controlling seasonal variations of dissolved inorganic carbon in the North Sea. Limnol Oceanogr 51:2746–2762

Bradshaw A, Brewer P, Shaffer D, Williams R (1981) Measurements of total carbon dioxide and alkalinity by potentiometric titration in the GEOSECS program. Earth Planet Sc Lett 55:99–115

Brand LE (1982) Genetic variability and spatial patterns of genetic differentiation in the reproductive rates of the marine coccolithophores *Emiliania huxleyi* and *Gephyrocapsa oceanica*. Limnol Oceanogr 27:236–245

Brand LE (1994) Physiological ecology of marine coccolithophores. In: Winter A, Siesser WG (eds.) Coccolithophores, Cambridge University Press, Cambridge, pp. 39–50

Brewer P, Bradshaw A, Williams R (1986) The changing carbon cycle - a global analysis. Springer, pp. 358–381

Broerse ATC, Ziveri P, Honjo S (2000) Coccolithophore (-$CaCO_3$) flux in the Sea of Okhotsk: seasonality, settling and alteration processes. Mar Micropaleontol 39:179–200

Bronk DA (2002) Dynamics of DON. In: Hansell DA, Carlson CA (eds.) Biogeochemistry of Marine Dissolved Organic Matter, Academic Press, London, pp. 153–247

Bronk DA, Lomas MW, Glibert PM, Schukert KJ, Sanderson MP (2000) Total dissolved nitrogen analysis: comparisons between the persulfate, UV and high temperature oxidation methods. Mar Chem 69:163–178

Brown CW, Yoder JA (1994) Coccolithophorid blooms in the global ocean. J Geophys Res 99:7467–7482

Brown L, Sanders R, Savidge G, Lucas CH (2003) The uptake of silica during the spring bloom in the Northeast Atlantic Ocean. Limnol Oceanogr 48:1831–1845

Bruland KW, Rue EL, Smith GJ, DiTullio G (2005) Iron, macronutrients and diatom blooms in the Peru upwelling regime: brown and blue waters of Peru. Mar Chem 93:81–103

Brzezinski MA, Nelson DM (1995) The annual silica cycle in the Sargasso Sea near Bermuda. Deep-Sea Res Part I 42:1215–1237

Burkhardt S, Amoroso G, Riebesell U, Sültemeyer D (2001) CO_2 and HCO_3^- uptake in marine diatoms acclimated to different CO_2 concentrations. Limnol Oceanogr 46:1378–1391

Burkhardt S, Riebesell U, Zondervan I (1999) Effects of growth rate, CO_2 concentration, and cell size on the stable carbon isotope fractions in marine phytoplankton. Geochim Cosmochim Acta 63:3729–3741

Capone DG, Knapp AN (2007) A marine nitrogen cycle fix? Nature 445:159–160

Carter CM, Ross AH, Schiel DR, Howard-Williams C, Hayden B (2005) In situ microcosm experiments on the influence of nitrate and light on phytoplankton community composition. J Exp Mar Biol Ecol 326:1–13

Cembella AD, Antia NJ, Harrison PJ (1984) The Utilization of Inorganic and Organic Phosphorus Compounds as Nutrients by Eukaryotic Microalgae: A Multidisciplinary Perspective: Part 1. Crit Rev Microbiol 10:317–391

Charlson RJ, Lovelock JE, Andreae MO, Warren SG (1987) Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate. Nature 326:655–661

Cho BC, Park MG, Shim JH, Azam F (1996) Significance of bacteria in urea dynamics in coastel surface waters. Mar Ecol Prog Ser 142:19–26

Claquin P, Martin-Jézéquel V, Kromkamp JC, Veldhuis MJW, Kraay GW (2002) Uncoupling of silicon compared with carbon and nitrogen metabolisms and the role of the

cell cycle in continuous cultures of *Thalassiosira pseudonana* (Bacillariophyceae) under light, nitrogen, and phosphorus control. J Phycol 38:922–930

Collier JL, Brahamsha B, Palenik B (1999) The marine cyanobacterium *Synechococcus* sp. WH7805 requires urease (urea amidohydrolase, EC 3.5.1.5) to utilize urea as a nitrogen source: molecular-genetic and biochemical analysis of the enzyme. Microbiology 145:447–459

Corstjens PLA, González EL (2004) Effects of nitrogen and phosphorus availability on the expession of the coccolith-vesicle V-ATPase (subunit *c*) of *Pleurochrysis* (Haptophyta). J Phycol 40:82–87

Cullen JJ, Yang X, MacIntyre HL (1992) Nutrient Limitation of Marine Photosynthesis. In: Falkowski PG, Woodhead AD (eds.) Primary Productivity and Biogeochemical Cycles in the Sea, Plenum Press, New York and London, pp. 69–88

Del Amo Y, Brzezinski MA (1999) The chemical form of dissolved Si taken up by marine diatoms. J Phycol 35:1162–1170

Delille B, Harlay J, Zondervan I, Jacquet S, Chou L, Wollast R, Bellerby GJ Richard, Frankignoulle M, Veiira Borges A, Riebesell U, Gattuso JP (2005) Response of primary production and calcification to changes of pCO_2 during experimental blooms of the coccolithophorid *Emiliania huxleyi*. Global Biogeochem Cy 19:doi:10.1029/2004GB002318

Denman KL, Brasseur G, Chidthaisong A, Ciais P, Cox PM, Dickinson RE, Hauglustaine D, Heinze C, Holland E, Jacob D, Lohmann U, Ramachandran S, da Silva Dias PL, Wofsy SC, Zhang X (2007) Couplings Between Changes in the Climate System and Biogeochemistry. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds.) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Dickson A, Millero F (1987) A comparison of the equilibrium constants for the dissociation of carbon acid in seawater media. Deep-Sea Research 34:1733–1743

Dittmar T, Fitznar HP, Kattner G (2001) Origin and biogeochemical cycling of organic nitrogen in the eastern Arctic Ocean as evident from D- and L-amino acids. Geochim Cosmochim Ac 65:4103–4114

DiTullio GR, Geesey ME (2002) Photosynthetic pigments in marine algae and bacteria. In: Bitton G (ed.) The Encyclopedia of Environmental Microbiology, John Wiley & Sons Inc., New York, New York, pp. 2453–2470

DiTullio GR, Smith Jr WO (1995) Relationship between dimethylsulfide and phytoplankton pigment concentration in the Ross Sea, Antarctica. Deep-Sea Res Part I 42:873–892

Dixon NE, Blakeley CGRL, Zerner B (1975) Jack Bean Urease (EC 3.5.1.5). A Metalloenzyme. A Simple Biological Role for Nickel? J Am Chem Soc 97:4131–4133

Dugdale (1967) Nutrient limitation in the sea: Dynamics, identification, and significance. Limnol Oceanogr 12:685–695

Dyhrman ST, Palenik B (2003) Characterization of ectoenzyme activity and phosphate-regulated proteins in the coccolithophore *Emiliania huxleyi*. J Plankton Res 25:1215–1225

Egge JK, Aksnes DL (1992) Silicate as regulating nutrient in phytoplankton competition. Mar Ecol Prog Ser 83:281–289

Egge JK, Heimdal BR (1994) Blooms of phytoplankton including *Emiliania huxleyi* (Haptophyta). Effects of nutrient supply in different N : P ratios. Sarsia 79:333–348

Eppley RW (1972) Temperature and Phytoplankton Growth in the Sea. Fishery Bulletin 70:1063–1085

Eppley RW, Harrison WG, Chisholm SW, Stewart E (1977) Particulate organic matter in surface waters off Southern California and its relationship to phytoplankton. J Mar Res 35:671–696

Eppley RW, Rogers JN, McCarthy JJ (1969) Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol Oceanogr 14:912–920

Esaias WE, Feldman GC, McClain CR, Elrod JA (1986) Monthly satellite-derived phytoplankton pigment distribution during the spring bloom in the North Atlantic Ocean. EOS: Transactions Am Geophys Union 67:835–837

Falkowski PG, Katz ME, Knoll AH, Quigg A, Raven JA, Schofield O, Taylor FJR (2004) The Evolution of Modern Eukaryotic Phytoplankton. Science 305:354–360

Falkowski PG, Laws EA, Barber RT, Murray JW (2003) Phytoplankton and Their Role in Primary, New, and Export Production. In: Fasham MJR (ed.) Ocean Biogeochemistry, Sringer-Verlag, Berlin Heidelberg New York

Falkowski PG, Raven JA (eds.) (1997) Aquatic Photosynthesis. Blackwell Sci., Malden, Mass., Berlin Heidelberg New York

Fan C, Glibert P, Alexander J, Lomas M (2003) Characterization of urease activity in three marine phytoplankton species, *Aureococcus anophagefferens*, *Prorocentrum minimum*, and *Thalassiosira weissflogii*. Mar Biol 142:949–958

Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science 305:362–366

Feng Y, Warner ME, Zhang Y, Sun J, Fu FX, Rose JM, Hutchins DA (2008) Interactive effects of increasing pCO_2, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae). Eur J Phycol 43:87–98

Föllmi KB (1996) The phosphorus cycle, phosphogenesis and marine phosphate-rich deposits. Earth Sci Rev 40:55–124

Fu FX, Warner ME, Zhang Y, Feng Y, Hutchins DA (2007) Effects of increased temperature and CO_2 on photosynthesis, growth and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). J Phycol 43:485–496

Fu FX, Zhang Y, Bell PRF, Hutchins DA (2005) Phosphate uptake and growth kinetics of *Trichodesmium* (Cyanobacteria) isolates from the North Atlantic Ocean and the Great Barrier Reef, Australia. J Phycol 41:62–73

Gaedeke A, Sommer U (1986) The influence of the frequency of periodic disturbances on the maintenance of phytoplankton diversity. Oecologia 71:25–28

García Ruiz R, Hernández I, Lucena J, Niell FX (1997) Preliminary studies on the significance of alkaline phosphatase activity in the diatom *Phaeodactylum tricornutum* Bohlin. Sci Mar 61:517–525

Geider RJ, La Roche J (2002) Redfield revisted: variability of C:N:P in marine microalgae and its biochemical basis. Eur J Phycol 37:1–17

Geider RJ, Osborne RA (1992) Algal Photosynthesis: The Measurement of Algal Gas Exchange. Chapman and Hall, Inc., New York

Gran G (1952) Determination of the equivalence point in potentiometric titrations of seawater with hydrochloric acid. Oceanol Acta 5:209–218

Grasshoff K, Kremling K, Ehrhardt M (eds.) (1999) Methods of Seawater Analysis. WILEY-VCH Verlag GmbH, Weinheim

Guillard R, Ryther J (1962) Studies of marine planktonic diatoms, I, *Cyclotella nanna* (Hustedt) and *Detonula convervacea* (Cleve). Can J Microbiol 8:229–239

Guillard RRL (1975) Culture of Phytoplankton for Feeding Marine Invertebrates. In: Smith WL, Chanley MH (eds.) Culture of Marine Invertebrate Animals, Plenum Press, New York, pp. 29–60

Hagino K, Okada H (2006) Intra- and infra-specific morphological variation in selected coccolithophore species in the equatorial and subequatorial Pacific Ocean. Mar Micropaleontol 58:184–206

Hansen HP, Koroleff F (1999) Determination of nutrients. In: Grasshoff K, Kremling K, Ehrhardt M (eds.) Methods of seawater analysis, Wiley-VCH, Weinheim, pp. 159–228

Hare CE, Ditullio GR, Trick CR, Wilhelm SW, Bruland KW, Rue EL, Hutchins DA (2005) Phytoplankton community structure changes following simulated upwelled iron inputs in the Peru upwelling region. Aquat Microb Ecol 38:269–282

Hare CE, Leblanc K, Ditullio GR, Kudela RM, Zhang Y, Lee PA, Riseman S, Hutchins DA (2007) Consequences of increased temperature and CO_2 for phytoplankton community structure in the Bering Sea. Mar Ecol Prog Ser 352:9–16

Harrison PJ, Davis CO (1979) The Use of outdoor Phytoplankton Continuous Cultures to Analyse Factors Influencing Species Selection. J Exp Mar Biol Ecol 41:9–23

Hays GC, Richardson AJ, Robinson C (2005) Climate change and marine plankton. Trends Ecol Evol 20:337–344

Hegarty SG, Villareal TA (1998) Effects of light level and N:P supply ratio on the composition between *Phaeocystis* cf. *pouchetii* (Hariot) Lagerheim (Prymnesiophyceae) and five diatom species. J Exp Mar Biol Ecol 226:241–258

Hein M, Sand-Jensen K (1997) CO_2 increases oceanic primary production. Nature 388:526–527

Heinze C, Maier-Reimer E, Winn K (1991) Glacial $p\mathrm{CO}_2$ reduction by the World Ocean: experiments with the Hamburg carbon cycle model. Paleoceanography 6:395–430

Holligan PM, Fernández E, Aiken J, Balch WM, Boyd P, Burkill PH, Finch M, Groom SB, Malin G, Muller K, Purdie DA, Robinson C, Trees CC, Turner SM, van der Wal P (1993) A Biogeochemical Study of the Coccolithophore, *Emiliania huxleyi*, in the North Atlantic. Global Biogeochem Cycles 7:879–900

Honjo S (1976) Coccoliths: production, transportation and sedimentation. Mar Micropaleontol 1:65–79

Hood RR, Laws EA, Armstrong RA, Bates NR, Brown CW, Carlson CA, Chai F, Doney SC, Falkowski PG, Feely RA, Friedrichs MAM, Landry MR, Moore JK, Nelson DM, Richardson TL, Salihoglu B, Schartau M, Toole DA, Wiggert JD (2006) Pelagic functional group modeling: Progress, challenges and prospects. Deep-Sea Res Part II 53:459–512

Houdan A, Probert I, Zatylny C, Veron B, Billard C (2006) Ecology of oceanic coccolithophores. I. Nutritional preferences of the two stages in the life cycle of *Coccolithus braarudii* and *Calcidiscus leptoporus*. Aquat Microb Ecol 44:291–301

Huang B, Hong H (1999) Alkaline Phosphatase Activity and Utilization of Dissolved Organic Phosphorus by Algae in Subtropical Coastal Waters. Mar Pollut Bull 39:205–211

Hutchins DA, DiTullio GR, Zhang Y, Bruland KW (1998) An iron limitation mosaic in the California upwelling regime. Limnol Oceanogr 43:1037–1054

Hutchins DA, Fu FX, Zhang Y, Warner ME, Feng Y, Portune K, Bernhardt PW, Mulholland MR (2007) CO_2 concentration controls nitrogen fixation rates of the marine cyanobacterium *Trichodesmium*. Limnol Oceanogr 52:1293–1304

Hutchins DA, Pustizzi F, Hare CE, DiTullio GR (2003) A shipboard natural community continuous culture system for ecologically relevant low-level nutrient enrichment experiments. Limnol Oceanogr Methods 1:82–91

Hutchinson GE (1961) The Paradox of the Plankton. Am Nat 95:137–145

Ietswaart T, Schneider P, Prins R (1994) Utilization of Organic Nitrogen Sources by Two Phytoplankton Species and a Bacterial Isolate in Pure and Mixed Cultures. Appl Environ Microbiol 60:1554–1560

Iglesias-Rodríguez MD, Brown CW, Doney SC, Kleypas J, Kolber D, Kolber Z, Hayes PK, Falkowski PG (2002) Representing key phytoplanton functional groups in ocean carbon cycle models: Coccolithophorids. Global Biogeochem Cycles 16:doi: 10.1029/2001GB001454

Jørgensen NOG (2006) Uptake of urea by estuarine bacteria. Aquat Microb Ecol 42:227–242

Karl DM, Björkman KM (2002) Dynamics of DOP. In: Hansell DA, Carlson CA (eds.) Biogeochemistry of Marine Dissolved Organic Matter, Academic Press, pp. 250–366

Keller MD, Bellows WK, Guillard RRL (1989) Dimethyl sulfide production in marine phytoplankton. In: Saltzman ES, Cooper WJ (eds.) Biogenic S in the Environment, Am. Chem. Soc., pp. 167–182

Kiene RP, Slezak D (2006) Low dissolved DMSP concentrations in seawater revealed by small-volume gravity filtration and dialysis sampling. Limnol Oceanogr: Methods 4:80–95

Kinnaer PR, Gray CD (1997) SPSS for Windows made simple. Hove: Psychology Press, second edition edn.

Kremling K, Wenck A, Pohl C (1987) Summer Distribution of Dissolved Cd, Co, Cu, Mn, and Ni in Central North Sea Water. Dt hydrogr Z 40:103–114

Kristiansen S (1983) Urea as a nitrogen source for the phytoplankton in the Oslofjord. Mar Biol 74:17–24

Kuenzler EJ, Perras JP (1965) Phosphatases of Marine Algae. Biol Bull 128:271–284

Lalli CM, Parsons TR (1994a) Energy flow and mineral cycling. In: Biological Oceanography: An Introduction, Pergamon, pp. 115–150

Lalli CM, Parsons TR (1994b) Phytoplankton and Primary Production. In: Biological Oceanography: An Introduction, Pergamon, pp. 45–79

Landry MR, Hassett RP (1982) Estimating the grazing impact of marine microzooplankton. Mar Biol 67:283–288

Langer G, Geisen M, Baumann KH, Kläs J, Riebesell U, Thoms S, Young JR (2006) Species-specific responses of calcifying algae to changing seawater carbonate chemistry. Geochem Geophys Geosys 7:Q09006, doi:10.1029/2005GC001227

Leftley JW, Syrett PJ (1973) Urease and ATP:Urea Aminolyase Activity in Unicellular Algae. Journal of General Microbiology 77:109–115

Leonardos N, Geider RJ (2005) Elevated atmospheric carbon dioxide increases organic carbon fixation by *Emiliania huxleyi* (Haptophyta), under nutrient-limited high-light conditions. J Phycol 41:1196–1203

Lewis E, Wallace D (1998) Program developed for CO2 system calculations ORNL/CDIAC-105. Tech. rep., Carbon Dioxide Information Analysis Centre, Oak Ridge National Laboratory, U.S. Department of Energy

Lochte K, Ducklow HW, Fasham MJR, Stienens C (1993) Plankton succession and carbon cycling at 47°N 20°W during the JGOFS North Atlantic Bloom Experiment. Deep-Sea Res Part II 40:91–114

Lohr M, Wilhelm C (1999) Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. Proc Natl Acad Sci USA 96:8784–8789

Lomas MW (2004) Nitrate reductase and urease enzyme activity in the marine diatom *Thalassiosira weissflogii* (Bacillariophyceae): interactions among nitrogen substrates. Mar Biol 144:37–44

Lomas MW, Glibert PM (1999) Interactions between NH_4^+ and NO_3^- uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures. Mar Biol 133:541–551

Lorrain A, Savoye N, Chauvaud L, Paulet YM, Naulet N (2003) Decarbonation and preservation method for the analysis of organic C and N contents and stable isotope ratios of low-carbonated suspended particulate material. Anal Chim Acta 491:125–133

Mackey DJ, O'Sullivan JE, Watson RJ, Dal Pont G (2002) Trace metals in the Western Pacific: temporal and spatial variability in the concentrations of Cd, Cu, Mn and Ni. Deep-Sea Res Part I 49:2241–2259

Maier-Reimer E, Mikolajewicz U, Winguth A (1996) Future ocean uptake of CO_2: interaction between ocean circulation and biology. Clim Dynam 12:711–721

Malin G, Stienke M (2004) Dimethyl production: What is the contribution of coccolithophores? In: Thierstein HR, Young JR (eds.) Coccolithophores: From molecular processes to global impact, Springer, Berlin, pp. 129–136

Martin-Jézéquel V, Hildebrand M, Brzezinski MA (2000) Silicon Metabolism in Diatoms: Implications for Growth. J Phycol 36:821–840

McCarthy JJ (1972) The uptake of urea by marine phytoplankton. J Phycol 8:216–222

McComb RB, Bowers GN, Posen S (1979) Alkaline phosphatase. Plenum Press, New York

Mehrbach C, Culberson C, Hawley J, Pytkovicz R (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr 18:897–907

Merrett MJ, Dong LF, Nimer NA (1993) Nitrate availability and calcite production in *Emiliania huxleyi* Lohmann. Eur J Phycol 28:243–246

Middelburg JJ, Nieuwenhuize J (2000) Nitrogen uptake by heterotrophic bacteria and phytoplankton in the nitrate-rich Thames estuary. Mar Ecol Prog Ser 203:13–21

Mobley HLT, Hausinger RP (1989) Microbial Ureases: Significance, Regulation, and Molecular Characterisation. Microbiol Rev 53:85–108

Moore J, Doney S, Kleypas J, Glover D, Fung I (2002) An intermediate complexity marine ecosystem model for the global domain. Deep-Sea Res Part II 49:403–462

Nelson D, Smith W, Muench R, Gordon L, Sullivan C, Husby D (1989) Particulate matter and nutrient distributions in the ice-edge zone of the Weddell Sea: relationship to hydrography during late summer. Deep-Sea Res 36:191–209

Nielsen MV (1995) Photosynthetic characteristics of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae) exposed to elevated concentrations of dissolved inorganic carbon. J Phycol 31:715–719

Nimer NA, Merrett MJ (1993) Calcification rate in *Emiliania huxleyi* Lohmann in response to light, nitrate and avaliability of inorganic carbon. New Phytol 123:673–677

Niraula MP, Casareto BE, Smith SL, Hanai T, Suzuki Y (2007) Examining the effects of nutrients on the composition and size of phytoplankton using unaltered deep-sea waters. J Exp Mar Biol Ecol 348:23–32

Oh SJ, Yamamoto T, Kataoka Y, Matsuda O, Matsuyama Y, Kotani Y (2002) Utilization of dissolved organic phosphorus by the two toxic dinoflagellates, *Alexandrium tamrense* and *Gymnodinium catentum* (Dinophyceae). Fisheries Sci 68:416–424

Oliveira L, Antia NJ (1986) Nickel Ion Requirements for Autotrophic Growth of Several Marine Microalgae with Urea Serving as Nitrogen Source. Can J Fish Aquat Sci 43:2427–2433

Olson RJ, Zettler ER, DuRand MD (1993) Phytoplankton analysis using flow cytometry. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds.) Handbook of methods in aquatic microbial ecology, Lewis Publishers, pp. 175–186

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner GK, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig MF, Yamanaka Y, Yool A (2005) Anthropogenic

ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–686

Ou L, Huang B, Lin L, Hong H, Zhang F, Chen Z (2006) Phosphorus stress of phytoplankton in the Taiwan Strait determined by bulk and single-cell alkaline phosphatase activity assays. Mar Ecol Prog Ser 327:95–106

Paasche E (1964) A tracer study of the inorganic carbon uptake during coccolith formation and photosynthesis in the coccolithophorid *Coccolithus huxleyi*. Physiol Plant Suppl 3:1–82

Paasche E (1998) Roles of nitrogen and phosphorus in coccolith formation in *Emiliania huxleyi* (Prymnesiophyceae). Eur J Phycol 33:33–42

Paasche E (2002) A review of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions. Phycologia 40:503–529

Paasche E, Brubak S (1994) Enhanced calcification in the coccolithophorid *Emiliania huxleyi* (Haptophyceae) under phosphorus limitation. Phycologia 33:324–330

Palenik B, Henson S (1997) The use of amides and other organic nitrogen sources by the phytoplankton *Emiliania huxleyi*. Limnol Oceanogr 42:1544–1551

Palenik B, Koke JA (1995) Charakterization of a Nitrogen-Regulated Protein Identified by Cell Surface Biotinylation of a Marine Phytoplankton. Appl Environ Microbiol 61:3311–3315

Palenik B, Morel FMM (1990) Comparison of cell-surface L-amino acid oxidases from several marine phytoplankton. Mar Ecol Prog Ser 59:195–201

Peers GS, Milligan AJ, Harrison PJ (2000) Assay optimization and regulation of urease activity in two marine diatoms. J Phycol 36:523–528

Platt TP, Gallegos CL (1980) Modeling primary production. In: Falkowski PG (ed.) Primary Productivity in the Sea, Ed. Plenum Press., pp. 339–351

Price NM, Morel FMM (1991) Colimitation of phytoplankton growth by nickel and nitrogen. Limnol Oceanogr 36:1071–1077

Raven JA, Johnston AM (1991) Mechanisms of inorganic carbon acquisition in marine phytoplankton and their implications for the use of other resources. Limnol Oceanogr 36:1701–1714

Rees TAV, Allison VJ (2006) Evidence for an extracellular L-amino acid oxidase in nitrogen-deprived *Phaeodactylum tricornutum* (bacillariophyceae) and inhibition of enzyme activity by dissolved inorganic carbon. Phycologia 45:337–342

Rees TAV, Bekheet IA (1982) The role of nickel in urea assimilation by algae. Planta 156:385–387

Riebesell U (2004) Effects of CO_2 enrichment on marine phytoplankton. J Oceanogr 60:719–729

Riebesell U, Zondervan I, Rost B, Tortell PD, Zeebe RE, Morel FMM (2000) Reduced calcifcation of marine plankton in response to increased atmospheric CO_2. Nature 407:364–367

Riegman R, Noordeloos AAM, Cadée GC (1992) *Phaeocystis* blooms and eutrophication of the continental coastal zones of the North Sea. Mar Biol 112:479–484

Riegman R, Stolte W, Noordeloos AAM, Slezak D (2000) Nutrient Uptake and Alkaline Phosphatase (EC 3:1:3:1) Activity of *Emiliania huxleyi* (Prymnesiophyceae) during Growth under N and P Limitation in Continuous Cultures. J Phycol 36:87–96

Robertson JE, Watson AJ, Langdon C, Ling RD, Wood JW (1993) Diurnal variation in surface pCO_2 and O_2 at 60°N, 20°W in the North Atlantic. Deep-Sea Res Part II 40:409–422

Rose JM, Feng Y, Gobler CJ, Gutierrez R, Hare CE, Leblanc K, Hutchins DA (2008) The Effects of Increased pCO_2 and Temperature on the North Atlantic Spring Bloom. II. Microzooplankton dynamics. Limnol Oceanogr submitted

Rost B, Riebesell U (2004) Coccolithophores and the biological pump: responses to environmental changes. In: Thierstein HR, Young JR (eds.) Coccolithophores, From Molecular Processes to Global Impact, Springer, Berlin, pp. 99–125

Rost B, Riebesell U, Burkhardt S, Sültemeyer D (2003) Carbon acquisition of bloom-forming marine phytoplankton. Limnol Oceanogr 48:55–67

Rousseeuw PJ, van Zomeren BC (1990) Unmasking multivariate outliers and leverage points (with discussion). J Am Stat Assoc 85:633–639

Saager PM, de Baar HJW, de Jorg JTM, Nolting RF, Schijf J (1997) Hydrography and local sources of dissolved trace metals Mn, Ni, Cu, and Cd in the northeast Atlantic Ocean. Mar Chem 57:195–216

Sáez AG, Probert I, Geisen M, Quinn P, Young JR, Medlin LK (2003) Pseudo-cryptic speciation in coccolithophores. P Natl Acad Sci USA 100:7163–7168

Saito MA, Goepferd TJ, Ritt JT (2008) Some thoughts on the concenpt of colimitation: Three definitions and the importance of bioavailability. Limnol Oceanogr 53:276–290

Sarmiento JL, Hughes TMC, Stouffer RJ, Manabe S (1998) Simulated response of the ocean carbon cycle to anthropogenic climate warming. Nature 393:245–249

Sarmiento JL, Slater R, Barber R, Bopp L, Doney AC, Kleypas J, Matear R, Mikolajewicz U, Monfray P, Soldatov V, Spall SA, Stouffer R (2004) Response of ocean ecosystems to climate warming. Global Biogeochem Cycles 18:GB3003, doi: 10.1029/2003GB002134

Sarthou G, Timmermans KR, Blain S, Tréguer P (2005) Growth physiology and fate of diatoms in the ocean: a review. J Sea Res 53:25–42

Schneider U (2004) Influence of carbonate chemistry and light intensity on natural phytoplankton assemblages with emphasis on species composition. Ph.D. thesis, Universität Bremen, Germany

Schoemann V, Becquevorta S, Stefelsb J, Rousseaua V, Lancelot C (2005) *Phaeocystis* blooms in the global ocean and their controlling mechanisms: a review. Journal of Sea Research 53:43–66

Sciandra A, Harlay J, Lefèvre D, Lemée R, Rimmelin P, Denis M, Gattuso JP (2003) Response of coccolithophorid *Emiliania huxleyi* to elevated partial pressure of CO_2 under nitrogen limitation. Mar Ecol Prog Ser 261:111–122

Shaked Y, Xu Y, Leblanc K, Morel FMM (2006) Zinc availability and alkaline phosphatase activity in *Emiliania huxleyi*: Implications for Zn-P co-limitation in the ocean. Limnol Oceanogr 51:299–309

Shi Y, Hu H, Ma R, Cong W, Cai Z (2004) Improved use of organic phosphate by *Skeletonema costatum* through relation of Zn^{2+} concentrations. Biotechnol Lett 26:747–751

Sieracki CK, Sieracki ME, Yentsch CM (1993) An imaging analysis system for automated analysis for marine microplankton. Mar Ecol Prog Ser 168:285–296

Sikes CS, Rober RD, Wilbur KM (1980) Photosynthesis and coccolith formation: Inorganic carbon sources and net inorganic reaction of deposition. Limnol Oceanogr 25:248–261

Smayda TJ (1980) Phytoplankton Species Succession. In: Morris I (ed.) The physiological ecology of phytoplankton, Blackwell, Oxford, pp. 493–570

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds.) (2007) IPCC, 2007: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Solorzano L, Sharp JH (1980) Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnol Oceanogr 25:756–760

Song B, Ward BB (2007) Molecular cloning and characterization of high-affinity nitrate transporters in marine phytoplankton. J Phycol 43:542–552

Steinke M, G M, Archer SD, Burkill PH, Liss PS (2002) DMS production in a coccolithophorid bloom: evidence for the importance of dinoflagellate DMSP lyases. Aquatic Microb Ecol 26:259–270

Stoll HM, Klaas CM, Probert I, Ruiz Encinar J, Alonso IG (2002) Calcification rate and temperature effects on Sr partitioning in cocoliths of multiple species of coccolithophorids in culture. Global Planet Change 34:153–171

Strathmann RR (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol Oceanogr 12:411–418

Thierstein HR, Young JR (eds.) (2004) Coccolithophores, From Molecular Processes to Global Impact. Springer, Berlin Heidelberg New York

Tilman DH (1982) Resource competition and community structure. Princeton Univ. Press

Tomas CR (1996) Identifying marine diatoms and dinoflagellates. Academic Press, San Diego

Tortell PD, DiTullio GR, Sigman DM, Morel FMM (2002) CO_2 effects on taxonomic composition and nutrient utilization in an Equatorial Pacific phytoplankton assemblage. Mar Ecol Prog Ser 236:37–43

Trimborn S, Langer G, Rost B (2007) Effect of varying calcium concentrations and light intensities on calcification and photosynthesis in *Emiliania huxleyi*. Limnol Oceanogr :in press

Tyrrell T, Merico A (2004) *Emiliania huxleyi*: bloom observations and the conditions that induce them. In: Thierstein HR, Young JR (eds.) Coccolithophores: From molecular processes to global impact, Springer, Berlin, pp. 75–97

Tyrrell T, Taylor AH (1996) A modelling study of *Emiliania huxleyi* in the NE Atlantic. J Marine Syst 9:83–112

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplanktonmethodik. Mitt int Ver Limnol 9:138

van Boeckel WHM (1991) Ability of *Phaeocystis* sp. to grow on organic phosphates: direct measurement and prediction with the use of an inhibition constant. J Plankton Res 13:959–970

Varela DE, Harrison PJ (1999) Effect of ammonium on nitrate utilization by *Emiliania huxleyi*, a coccolithophore from the oceanic northeastern Pacific. Mar Ecol Prog Ser 186:67–74

Volk T, Hoffert MI (1985) Ocean carbon pumps: analysis of relative strengths and efficiencies in ocean-driven atmospheric CO_2 changes. In: Sunquist ET, Broecker WS (eds.)

The carbon cycle and atmospheric CO_2: natural variation archean to present, American Geophysical Union, Washington, D.C., Geophysical Monographs 32, pp. 99–110

Walz PM, Friederich GE (1996) Rapid automated analysis of total dissolved inorganic carbon and its application in the central California upwelling system during the CoOP95 experiment. EOS 76:OS 102

Waser NA, Yin K, Yu Z, Tada K, Harrison PJ, Turpin DH, Calvert SE (1998) Nitrogen isotope fractionation during nitrate, ammonium and urea uptake by marine diatoms and coccolithophores under various conditions of N availabitity. Limnol Oceanogr 169:29–41

Wassmann P, Ratkova T, Reigstad M (2005) The contribution of single and colonial cells of *Phaeocystis pouchetii* to spring and summer blooms in the north-eastern North Atlantic. Harmful Algae 4:823–840

Weeks AR, Fasham MJR, Aiken J, Harbour DS, Read JF, Bellan I (1993) The spatial and temporal development of the spring bloom during the JGOFS North Atlantic Bloom Experiment, 1989. J MAR BIOL ASSOC UK 73:253–282

Welschmeyer NA (1994) Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnol Oceanogr 39:1985–1992

Westbroek P, Brown CW, van Bleijswijk J, Brownlee C, Brummer GJ, Conte M, Egge J, Fernández E, Jordan R, Knappertsbusch M, Stefels J, Veldhuis M, van der Wal P, Young J (1993) A model system approach to biological climate forcing. The example of *Emiliania huxleyi*. Global Planet Change 8:27–46

White RH (1982) Analysis of dimethylsulfonium compounds in marine algae. J Mar Res 40:529–536

Winter A, Jordan RW, Roth PH (1994) Biogeography of living coccolithophores in ocean waters. In: Winter A, Siesser WG (eds.) Coccolithophores, Cambridge University Press, Cambridge, pp. 161–177

Wolf-Gladrow DA, Riebesell U, Burkhardt S, Bijma J (1999) Direct effects of CO_2 concentration on growth and isotopic composition of marine plankton. Tellus 51B:461–476

Xu Y, Wahlund TM, Feng L, Shaked Y, Morel FMM (2006) A novel alkaline phosphatase in the coccolithophore *Emiliania huxleyi* (Prymnesiophyceae) and its regulation by phosphorus. J Phycol 42:835–844

Yamaguchi H, Yamaguchi M, Fukami K, Adachi M, Nishijima T (2005) Utilization of phosphate diester by the marine diatom *Chaetoceros ceratosporus*. J Plankton Res 27:603–606

Yamashita Y, Tanoue E (2003) Distribution and alteration of amino acids in bulk DOM along a transect from bay to oceanic waters. Mar Chem 82:145–160

Young JR (1994) Functions of coccoliths. In: Winter A, Siesser WG (eds.) Coccolithophores, Cambridge University Press, Cambridge, pp. 63–82

Young JR, Davis SA, Bown PR, Mann S (1999) Coccolith Ultrastructure and Biomineralisation. J Struct Biol 126:195–215

Young JR, Ziveri P (2000) Calculation of coccolith volume and its use in calibration of carbonate flux estimates. Deep-Sea Res Part II 47:1679–1700

Zapata M, Rodriguiz F, Garrido JL (2000) Seperation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C_8 column and pyridine-containing mobile phases. Mar Ecol Prog Ser 195:29–45

Zeebe RE, Wolf-Gladrow DA (2001) CO_2 in seawater: equilibrium, kinetics, isotopes. Elsevier Science, Amsterdam

Ziveri P, Baumann KH, Böckel B, Bollmann J, Young JR (2004) Biogeography of selected Holocene coccoliths in the Atlantic Ocean. In: Thierstein HR, Young JR (eds.) Coccolithophores From Molecular Processes to Global Impact, Springer, London, pp. 403–428

Ziveri P, Broerse ATC, van Hinte JE, Westbroek P, Honjo S (2000) The fate of coccoliths at 48N 21W, northeastern Atlantic. Deep-Sea Res Part II 47:1853–1875

Ziveri P, de Bernardi B, Baumann KH, Stoll HM, Mortyn PG (2007) Sinking of coccolith carbonate and potential contribution to organic carbon ballasting in the deep ocean. Deep-Sea Res Part II 54:659–675

Zondervan I (2007) The effects of light, macronutrients, trace metals and CO_2 on the production of calcium carbonate and organic carbon in coccolithophores—A review. Deep-Sea Res Part II 54:521–537

Zondervan I, Rost B, Riebesell U (2002) Effect of CO_2 concentration on the PIC/POC ratio in the coccolithophore *Emiliania huxleyi* grown under light-limiting conditions and different daylengths. J Exp Mar Biol Ecol 272:55–70

Zondervan I, Zeebe RE, Rost B, Riebesell U (2001) Decreasing marine biogenic calcification: A negative feedback on rising atmospheric pCO_2. Global Biogeochem Cycles 15:507–516

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